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## 1982 NASA Space Biology Accomplishments

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25th Anniversary  
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# 1982 NASA Space Biology Accomplishments

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## Preface

The Space Biology Program currently includes forty-five research projects. The goals, objectives, accomplishments, and future plans of each project are described in this publication as individual technical summaries.

The intent in compiling this publication is twofold. First, we would like to provide the scientific community with an annual summary of the accomplishments resulting from research pursued under the auspices of NASA's Space Biology Program. Secondly, we hope to stimulate the exchange of information and ideas among scientists working in the Program. To facilitate this exchange process, a list of publications has been included with each project summary.

We would like to thank all the participants in the Space Biology Program for their cooperative response to our requests for information. We would also like to thank April Roy and Chris Bolcik for their technical assistance in preparing this report.

Thora W. Halstead  
May 1983



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## INTRODUCTION



## THE NASA SPACE BIOLOGY PROGRAM

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### Introduction

One of the major features of the physical environment on the surface of the Earth is the constant presence of the force of gravity. Terrestrial gravity has important biological consequences for the living organisms of the Earth. Among the effects of spaceflight, the phenomenon of weightlessness, therefore, provides the greatest biological research opportunity both because of its uniqueness to space and because of the importance of gravity to life on Earth. Access to space provides an opportunity to manipulate gravity from its norm of one down to almost zero, effectively providing the full spectrum of gravitational research capability for the first time. This capability, combined with the stability and pervasiveness of gravity on Earth, its obvious impact on biological evolution, and its continuing effect on the morphology, physiology, and behavior of living organisms, has led the Space Biology Program to concentrate its efforts and resources on investigating the biological significance of gravity.

### Program Goals

The goals of the Space Biology Program are to: use the unique characteristics of the space environment, particularly microgravity, as a tool to advance knowledge in the biological sciences; understand how gravity has shaped and affected life on Earth; and understand how the space environment affects both plant and animal species.

### Program Scope

The research in the Space Biology Program is divided into three broad areas:

1. Gravity receptor mechanisms. This includes the identification of the organ or site of gravity reception and the biological systems and mechanisms that transmit the information to a responsive site.
2. The physiological effects of gravity. This includes the use of gravity's physiological effects to explore biological problems; an understanding of how gravity affects and controls the physiology, morphology, and behavior of organisms; how gravity and other environmental stimuli and stresses interact in this control; and the biological mechanism by which living systems respond and adapt to altered gravity, particularly that of the space environment.
3. The role of gravity in development. Specifically, the effect of gravity on reproduction, development, maturation, and evolution.

#### Research Opportunities

With the proven feasibility of the Space Shuttle, we now have capability of performing biological experiments in space. The opportunity has arrived to use the locker space within the Shuttle orbiter on a continuing space available basis. This will provide a valuable augmentation to the ongoing ground-based research program.

Spaceflight will provide the validation for many experimental hypotheses developed in ground-based research, while gravitational experiments on Earth will continue to hone the questions, provide the necessary baseline data, and develop spaceflight experimental protocol.

The experimental approach of the ground-based studies in the Space Biology Program is to manipulate gravity on Earth and develop weightless simulation models to: (1) develop and test gravitational hypotheses, (2) identify gravity-sensitive biological systems and interacting environmental response mechanisms, (3) analyze biological systems and mechanisms known to be gravity-sensitive, (4) analyze flight experiment data and iteratively expand ground research capability, and (5) plan and design future space experiments. In addition, research is conducted to understand how the uncontrollable biodynamic factors of the spacecraft will affect the results of the various flight experiments.

Weightlessness, and physiological conditions similar to those induced by weightlessness, have traditionally been partially simulated in the ground-based laboratory by a variety of techniques.

In animal research, the attempt is to simulate the reduction in apparent weight and alteration in hydrostatic pressure that

occurs under weightless conditions. Simulation is accomplished in the Program's research by the use of a unique animal support system that reduces loading on the hind limbs and produces a cephalad fluid shift. This gentle and delicate method has been developed, as well as used, by scientists associated with the Space Biology Program and has been shown to successfully induce some of the physiological changes associated with weightlessness.

The clinostat is an apparatus which, by its motions, modifies or equalizes the directional input from gravity to an attached organism. The rotation of experimental material on a clinostat is useful only for those physiological phenomena that have relatively long exposure thresholds (or presentation times), so that it is possible to rotate the organism slowly enough to avoid the complications produced by centrifugal acceleration, but fast enough so that the time spent in any one position does not result in an induced georesponse.

To achieve hypergravity conditions, gravity loads above one are produced by slow centrifugation and are especially useful in identifying gravity-sensitive biological systems and mechanisms. Changing the directional input of gravity to an organism has also proved to be a successful technique to study the biological effects of gravity.

#### Present Focus of Program

The research focus of the Space Biology Program is dependent upon several dynamic factors: NASA requirements, the characteristics of flight experiment opportunities, the sensitivity of specific biological systems to gravity, the scientific value of the research, the state of knowledge and technology in the specific scientific areas, the interest of scientists in studying the biological questions, and the availability of funds to support the research.

Within the scope of the Space Biology Program, the current Program is focused on answering the following basic scientific questions:

1. What are the components of the gravity-sensing mechanisms of plants and animals? How do they perceive information? How is the information transmitted to evoke responses?
2. Does gravity influence plant and animal fertilization and development, and can fertilization and development proceed normally in a near zero gravity environment? If gravity does affect fertilization and development, what are the sensitive physiological systems and how are they affected? If early development is affected by gravity, is it a result of an effect on the parent or the direct effect on the embryo itself?

3. What is the role of gravity in the formation of structural elements such as lignin, cellulose, silica, chitin, and bone calcium phosphates at the molecular level as well as at more complex organizational levels?
4. What role does gravity play in calcium-mediated physiological mechanisms and in calcium metabolism?
5. How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology, and behavior of organisms? Or, how do gravitational and other environmental stimuli interact in their control and direction of living forms? Can the action of gravity be replaced by different stimuli?

#### Future Focus of Program

As longer flight missions become available, biological questions that require longer periods of micro-G, such as multigeneration and radiation experiments, will increase in importance. It has been recognized for some time that ionizing radiation causes cumulative damage in all living things, but knowledge of the biological effects of the cosmic radiation encountered in space, which contains high energy, heavy particles, is poorly understood. Furthermore, the biological consequences of the interaction of this unique radiation and gravity are less known. Since long-term space exposure is needed to adequately investigate the effects of radiation, such research is well suited for the space flights that a space station will provide.



## ANIMAL PROJECTS



## ALTERATIONS IN GUT TRANSPORT OF MINERALS AND IN BINDING PROTEINS DURING SIMULATED WEIGHTLESSNESS

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### SUMMARY

The objectives of this research program are: (1) to establish an animal model on Earth by which the effects of weightlessness on bone mineral homeostasis can be studied (i.e., simulated weightlessness), (2) to determine whether simulated weightlessness alters intestinal calcium transport, (3) to determine whether simulated weightlessness has a generalized or selective effect on bone mineral content, and (4) to determine whether changes in dietary calcium or hormone levels can modulate the effects of simulated weightlessness on bone mineral homeostasis.

The original rat support model (employing a back harness) has been modified by changing the point of support to the base of the tail. The rats are supported at a 40° angle such that their forepaws continue to bear weight but their hind paws do not. Such rats appear to be relatively unstressed. They gain weight continuously and at the same rate as pair-fed controls. Support times of 15 days are readily achieved. Besides providing healthy, happy, unstressed rats, this support system provides an important internal control for our studies--the hindlimbs are unweighted, the forelimbs are not. Thus comparison of the effects on calcium content in the loaded humerus (forelimb bone) and the unloaded tibia (hindlimb bone) will help determine whether this model of weightlessness produces generalized or local effects on bone.

Measurement of intestinal calcium transport by several different techniques revealed no alterations using this model of simulated weightlessness. When dietary calcium was altered to provide a range of very low calcium to very high calcium content, the absorption of calcium changed appropriately and similarly in both the supported and pair-fed control rats; rats fed a low calcium diet had a greater ability to absorb calcium than rats fed the high calcium diet regardless of whether they were supported.

When the calcium content of a variety of bones from the supported rats was compared with the calcium content in the same bones in pair-fed controls, several points became clear: (1) Supported rats had a significantly lower calcium content only in the unweighted bones such as the tibia and lumbar vertebra compared with pair-fed controls. The normally weighted bones such as the

humerus and mandible did not show differences between supported and control rats. (2) The differences in bone calcium content between supported and control rats was progressive with time of support; no significant differences were seen after 5 days, whereas the differences were greatest at 15 days (the longest time evaluated). (3) Despite the progressive disparity in bone calcium content between the tibia and lumbar vertebra of supported rats compared with control rats, calcium uptake by such bones showed a biphasic pattern; e.g., initially (2 to 5 days), and by 15 days, calcium uptake had rebounded to higher than control values. These data suggest an initial inhibition of bone formation followed by an increase in bone formation possibly secondary to increased bone resorption when the rats were initially supported.

The effects of dietary calcium on bone mineral content was studied in supported and pair-fed control rats. The following observations were made: (1) The calcium content of bone was directly proportional to the calcium content of the diet in both supported and pair-fed controls. Rats fed the very low calcium diet had very fragile bones regardless of whether they were or were not supported. (2) The differences between the unweighted bones of the supported and pair-fed control rats appeared to decrease as the dietary calcium content was raised. Thus, it is conceivable from these data that the bone losses occurring during spaceflight can be reduced or eliminated by increasing dietary calcium intake.

#### PUBLICATIONS

Bikle, D.D., Globus, R.K., and Morey, E.R. Calcium Transport from the Intestine and into Bone in a Rat Model Simulating Weightlessness. Physiologist 25(6, Suppl.): S143-S144, 1982.

Bikle, D.D. and Herman, R.H. Calcium Potentiates the Cyclic Nucleotide and Phosphaturic Response to Parathyroid Hormone Infusion. Journal of Clinical Endocrinology and Metabolism 56: 11-17, 1983.

## WEIGHTLESSNESS SIMULATION: PHYSIOLOGICAL CHANGES IN FAST AND SLOW MUSCLE

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### SUMMARY

This is a new project. In recent years there has been an increased interest in the adaptation of muscle to disuse, in particular to changes that occur with weightlessness during spaceflights. This laboratory will examine the effect of simulated weightlessness on the biochemical characteristics of fast and slow muscle alterations in the rat. In the model to be used, the rat will be supported so that the hindlimbs are unweighted, the head will be tilted downward, and all body weight will be borne by the forepaws which will also be used for locomotion.

During hypokinesia, biochemical parameters will be investigated in slow muscle, such as the soleus, and in fast muscle, such as the extensor digitorum longus. Of particular interest is the effect of disuse on the control of acetylcholinesterase (AChE) and its molecular forms. Other proteins to be investigated are those involved in neuromuscular transmission such as cholineacetyltransferase (CAT) and the acetylcholine receptor. In addition, the effects of weightlessness on axoplasmic transport of AChE and CAT and on the rate of denervation- and reinnervation-induced changes will be studied. The period of disuse will vary between 1 to 5 weeks followed by a another 1 to 5 week period to study the readaptation of the rat to normal conditions.

# MORPHOLOGICAL AND HISTOCHEMICAL STUDIES OF BONE AND CARTILAGE DURING PERIODS OF SIMULATED WEIGHTLESSNESS

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## SUMMARY

The primary objective of this study is to determine how weightless conditions influence the metabolism of skeletal tissues. When disuse of the skeleton occurs, such as during prolonged bed rest, or during low gravity or weightless periods, the skeleton undergoes a loss of integrity and mechanical strength. Development of animal models to mimic these conditions has been difficult, but the model provided by Emily Morey-Holton shows that rats exposed to 2 to 3 weeks of non-weight-bearing in a head-down position undergo bone reduction in a manner similar to animals subjected to spaceflight conditions. This lab has applied a combination of techniques using electron and light microscopy, histochemistry, and quantitative enzymology to determine how the skeletal tissues respond to this model system.

Alkaline phosphatase activity has been classically associated with the rate of new bone formation in all skeletal tissues. When this lab quantitated this enzyme activity from femurs of non-weight-bearing animals, there was a slight but statistically significant reduction in this activity in metaphyseal bone. However, histochemical methods for alkaline phosphatase show that this enzyme is found: (1) within the bone-forming osteoblast population, (2) within the calcifying cartilage of the femur, (3) along the fibrous components of the periosteum, and (4) within the cytoplasm of the polymorphonuclear leucocytes. Are all of these sites responding to the experimental treatment? By combining microscopy and histochemistry this lab is accumulating evidence that the osteoblasts (on bone surfaces) and fibroblasts (along the periosteum) are the major sites affected by the non-weight-bearing condition.

Electron microscopy of the fibrous sheath surrounding bone (i.e., the periosteum) has resulted in unexpected findings. First, special "gap" or communicating junctions between the cells of this fibrous tissue are reduced in numbers and size as a result of the reduced weight-bearing. This communicating system of junctions also occurs in bone and may function as a "nervous system" between connective tissue cells which directs the synchronous activity of these cells. Secondly, the periosteal fibroblasts appear to be resorbing the collagen fibers of the periosteum, indicating that the mechanical stress on the periosteum has been affected by the non-weight-bearing. This suggests that variation in mechanical stress probably affects all components of the musculoskeletal system.

## PUBLICATION

Doty, S.B. and Morey-Holton, E.R. Changes in Osteoblastic Activity Due to Simulated Weightless Conditions. Physiologist 25(6, Suppl.): S141-S142, 1982.

## REGULATION OF HEMATOPOIESIS IN THE RAT WEIGHTLESSNESS SIMULATION MODEL

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### SUMMARY

A decrease in the number of circulating red blood cells appears to be consistent sequelae of spaceflight. Likewise, changes in the number and function of white blood cells have been frequently reported. The cause(s) of these alterations is unknown and the biological significance is uncertain. The objective of the studies supported by this grant is to investigate the regulation of blood cell production in rats exposed to antiorthostatic hypokinesia. Data from these investigations will be added to the other information derived from antiorthostatic hypokinesic rats that suggests this system is a good model for the bone, muscle, and cardiovascular effects of spaceflight.

The approach to these investigations can be divided into two distinct sections. The first phase of the study is concerned with data collection to ascertain if the model simulates the hematopoietic effects of spaceflight. The second phase will be directed at mechanistic considerations.

The major accomplishment of the 1982 research has been to validate antiorthostatic hypokinesia in the rat as a surrogate for the zero gravity of space. Thus, from the hematological viewpoint the investigations have shown:

1. A biphasic change in body weight. A rapid loss occurs during the first 24 hours of support followed by a more protracted phase of either zero growth or continued loss of body weight.
2. Reduced food and water consumption.
3. Transient hemoconcentration, i.e., reduction in the plasma component of the blood.
4. Reduced production of red blood cells.
5. Decrease in average size of the red blood cells.
6. Reduced blood volume, a large part of which occurs in the first 24 hours of support.
7. Reduced number of circulating red blood cells, i.e., "anemia."



8. A postsupport increase in the number of circulating white blood cells.

All of the above changes are directly comparable with available data from humans and rats subjected to spaceflight. In addition, we have also shown functional changes in the hemoglobin in the red blood cells of supported rats. Such changes have been predicted to occur during spaceflight but have not yet been documented. Further, the lab's data are consistent and do not seem to be markedly dependent on the mechanism of support or on the site from which the blood samples are obtained.

It is concluded from these investigations that rats exposed to antiorthostatic hypokinesia show a variety of hematological effects directly comparable with those that occur in humans and rats exposed to spaceflight. Thus, antiorthostatic hypokinesia appears to be validated as a useful paradigm for zero gravity in which to investigate the hematological effects of spaceflight.

#### PUBLICATION

Dunn, C.D.R., Johnson, P.C., and Leach, C.S. Fluid Shifts and Erythropoiesis: Relevance to the "Anemia" of Space Flight. Physiologist 25(6, Suppl.): S79-S80, 1982.

# EFFECTS OF GRAVITO-INERTIAL FIELDS ON THE PHYSIOLOGY OF THE ORB-WEAVING SPIDER

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## SUMMARY

Mechanical receptors for the detection of gravity stimuli have evolved to respond to linear and/or angular acceleration. Perhaps the most complex system of gravity (G) receptors is seen among the vertebrates in which the utricular and semicircular canal systems have become exquisitely dedicated to gravito-inertial forces. Among the invertebrates, a progenitor of the linear acceleration detector can be seen in the statocyst. Statocysts (e.g., in the crayfish) employ a mass within an enclosed cavity such that changes in linear acceleration or position stimulate different hair cells. Spiders do not present such organs. A different receptor mechanism evolved to inform the animal about its orientation in the gravito-inertial field.

A primary objective of this research project is to expose the possible mechanism for gravity detection in the spider and to describe its operating characteristics. During the 1982 research year, it was demonstrated that the G function is logarithmic having an estimated absolute threshold of perhaps  $1.0008 G_z$ . This surprisingly small increment to normal gravity is tentatively explained by the exquisite sensitivity of the lyriform organ on the spider leg. Other research has shown that the lyriform can respond to mechanical displacement on the order of millimicrons. In this work, the gravity sensitivity of the spider is determined by examining the change in its heart rate following centrifugation.

During these first experiments it had often been observed that the vigor of the spider heart pulse changed with changes in G and with changes in tilt (i.e., a change in the direction of G). During the latter part of 1982 a tilt-system was designed and computer programs were written to analyze the amplitude density of the spider pulse as a function of tilt. At the time of this writing there is initial evidence to suggest a functional relationship between tilt and amplitude density of the pulse. It can be demonstrated that the spider's absolute sensitivity to tilt may be in the region of 2 degrees or less.

This growing knowledge about the spider gravity response system has suggested a model in which spiders detect the local G vector according to strains in their exoskeletons. Reflex mechanisms of the heart respond to this sensory input to produce a blood

pressure change in the legs. In this fashion the spider may be able to maintain postural homeostasis when either the magnitude or the direction of the G field changes.

#### PUBLICATION

Finck, A. Gravito-inertial Sensitivity of the Spider; Araneus sericatus. Physiologist 25(6, Suppl.): S121-S122, 1982.

## THERMOREGULATION IN PRIMATES IN HYPERACCELERATION FIELDS

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### SUMMARY

Three experimental studies have been performed since this program started slightly less than 12 months ago. These studies are examining the responses of a small diurnal primate, the squirrel monkey, to exposures to hypergravic fields. The first two of these studies involved examinations of acute exposures to 2 and 3G<sub>z</sub> fields. The third study examined responses to chronic exposure to 1.5 and 2G<sub>z</sub>.

In the first study, an examination of the thermoregulatory responses of the squirrel monkeys to 70-minute 2G<sub>z</sub> exposures, both during the day and night, was made. The response of the animal during the day was demonstrated to be a 1.5° C fall in colonic temperature, mediated at least in part by an increase in vasomotor heat loss. This response was highly reproducible and seen in all animals. In contrast, at night the temperature response was completely eliminated. Individual animals showed small deviations in temperature either in an upward or downward direction but on an average there was no change in colonic temperature. Similarly, the skin temperatures did not show any major deviations in vasomotor heat loss. These results demonstrate that although the response in body temperature is very prominent and reproducible during the day, physiologically these animals have the ability to regulate their body temperatures independent of this gravitational force during the night.

This day-night difference in a series of rats was also investigated. Historically, all rats exposed to hyper-gravitational fields have been examined during the day, which is the rest phase of these nocturnal animals. The investigators wanted to determine whether the light vs. the dark had a significant influence on the response or whether the magnitude of the response was the function of the activity phase vs. the rest phase of the animal. The previously reported 2° C response was observed when centrifuging the animals during the day. However, upon exposure to 2G<sub>z</sub> at night, which is the animal's active phase, the body temperature fall was even larger. This indicates that although the monkeys were able to conserve body temperature more readily than the rats, both species showed an increased ability to maintain body temperature during their rest phase when their circadian body temperatures were at a minimum.

The second study examined the primate sleep responses to 70-minute exposures to hypergravic fields. The investigators had previously noted that centrifuged animals, when observed visually, demonstrated a sleeping behavior periodically when in a hypergravic field. This study further examined this observation with electrophysiological recording of the EEG of a group of primates. It was noted that during a precentrifugation phase, the animals showed various amounts of "napping" behaviors in which slow-wave sleep was noted to occur on a periodic basis. (Rapid eye movement sleep was not observed during this daytime study.) Upon centrifugation, however, the amount of sleep was inhibited markedly for several minutes after which the slow-wave sleep pattern began to recur. The amount of sleep never reached the baseline level in the precentrifugation phase. Post-centrifugation, the amount of sleep increased significantly and was noted to be maintained for the entire 70-minute postcentrifugation phase. This response was relatively proportional in the animals studied at both 2 and 3G<sub>z</sub>. At 3G<sub>z</sub>, the animals showed an increased response such that there was a greater reduction in slow-wave sleep during the centrifugation phase, and an increased amount of slow-wave sleep during the postcentrifugation phase. It is interesting to note that the amounts of slow-wave sleep have always been inversely correlated with the level of body temperature. That is, lower body temperatures are usually correlated with increased sleep. In this experiment, this correlation did not hold since during centrifugation the amount of sleep observed during the fall in body temperature was less than the precontrolled phase where body temperature was higher.

The third study was an examination of the circadian response of these primates to chronic exposure of 1.5 and 2G<sub>z</sub>. In this experiment, the animals were maintained on the centrifuge for 90 days at different G levels. On the centrifuge with the animals was a small microcomputer which monitored the animals' feeding and drinking responses in half-hour intervals throughout this period of time. The animals were initially exposed to 1G for 3 weeks followed by 1.5G for approximately 2 weeks. This was followed by 2G for 5 weeks and then the animals were returned to 1G for the remainder of the study. In each phase, the animals were exposed to a normal 24-hour light-dark cycle. Additionally, during the precentrifugation and postcentrifugation 1G phases and during the 2G phase, the animals were exposed to constant light where the circadian system was allowed to free-run. In all instances, the animals maintained the normal 24-hour circadian rhythm when exposed to the light-dark cycle. There is some suggestion, however, that the synchronization was not as precise in the hypergravic fields. Further, these preliminary data suggest that there may have been a period change in the free-running rhythm at 2G. Further work needs to be performed to determine the exact responses.

## PUBLICATIONS

Fuller, C.A. and Williams, B.A. Primate Thermal Sensitivity to Short Hyperacceleration Profiles (Abstract). Physiologist 25(4): 231, 1982.

Fuller, C.A. and Williams, B.A. Short Hyperdynamic Profiles Influence Primate Temperature Regulation. Physiologist 25(6, Suppl.): S91-S92, 1982.

## CHANGES IN BONE STRUCTURE AND METABOLISM DURING SIMULATED WEIGHTLESSNESS: ENDOCRINE AND DIETARY FACTORS

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### SUMMARY

Bone mineral is lost from both the rat and human skeletons under conditions of weightlessness that occur during spaceflight. This loss in turn weakens the skeleton, increasing the likelihood of bone fractures. In addition, the disruption of normal mineral metabolism may adversely affect renal function.

The long-term objective of this project is to determine the cause of this mineral loss and at the same time investigate the dynamics of bone metabolism. The present goal is to examine the roles of vitamin D, parathyroid hormone (PTH), stress (corticosterone), and dietary calcium and phosphorus in the skeletal alterations induced by weightlessness. In this study the ground-based rat model developed at NASA Ames Research Center is used to simulate the weightlessness of spaceflight.

This program was initiated in August 1982. At present this lab is in the process of completing the first series of experiments designed to examine the relationships between dietary calcium and phosphorus, the bone changes induced by simulated weightlessness and the serum concentrations of calcium, inorganic phosphate, PTH, and the vitamin D metabolites. Four separate experiments have been completed utilizing different dietary levels of calcium and phosphorus. Measurement of the serum concentrations of calcium and inorganic phosphate and total bone ash of the tibia and humerus have been completed. Moreover, approximately 50% of the bone histomorphometric measurements designed to identify the specific changes in bone structure and metabolism associated with simulated weightlessness have been completed. Measurement of the serum concentrations of PTH and the vitamin D metabolites is underway.

The results of these initial experiments indicate that the bone mineral loss that occurs during simulated weightlessness on a diet with normal levels of calcium and phosphorus can be reduced by increasing the dietary intake of calcium and phosphorus. On a normal diet, tibia from animals undergoing simulated weightlessness showed a highly significant ( $p < .005$ ) 13% decrease in total bone weight when compared with tibia from control animals. On a high calcium/phosphorus diet no significant differences in bone weight were observed. When the measurements of PTH and the vitamin D metabolites are completed,

it should be possible to relate the apparent protective effect of increased dietary calcium and phosphorus to the primary hormones regulating mineral metabolism. Once this has been accomplished, the next plan is to determine whether or not PTH, either directly or indirectly, is mediating the bone mineral loss that occurs under normal dietary conditions.



## THERMOREGULATION IN RATS: EFFECTS OF HYPERGRAVIC FIELDS

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### SUMMARY

Experiments were completed to further characterize neural processing of sensory information under conditions of altered gravity. One set of experiments provided data on the thermoregulatory system of rats exposed to hypergravic fields. A second set of experiments involved measuring auditory brainstem responses in rats exposed to hypergravic fields. These experiments provide complementary information on how altered gravitational fields affect information processing involving the brainstem and hypothalamic areas of the central nervous system. In addition, the results of the experiments serve as the basis for future studies in hypogravic fields, particularly experiments on a space station.

A major objective during 1982 was to further elucidate the mechanisms underlying the impairment of temperature regulation in mammals exposed to hypergravic fields. Experiments involved measurement of the rate of oxygen consumption and core and tail temperatures in cold-exposed rats from 1 to 6G. To briefly summarize the results of the studies, the data were consistent with the proposal that the thermoregulatory system is composed of a parallel arrangement of neural controllers that can be reversibly uncoupled by placing the animal in a hypergravic field. In hypergravic fields, the parallel controllers for both modes of heat production (shivering and nonshivering thermogenesis) fail to adequately activate thermogenic effectors, even though these parallel controllers for thermogenesis are responsive to lowered ambient temperature. Moreover, acclimation to cold prior to acute cold exposure does not prevent the decrease in core temperature seen in animals acclimated to an ambient temperature of 23°C. Similar experiments in a hypogravic environment would serve to characterize a mammalian neural regulatory system over a range of 0 to 1G.

A secondary objective was to determine the effects of hypergravity on auditory brainstem responses (ABRs). It was found that hypergravic fields of 6G, but not 3G, altered the ABRs. The ABRs have a series of negative peaks (1N, 2N,....) and positive peaks (1P, 2P,....) and at 6G there were significant increases in central conduction time for peaks 3N, 4P, 4N, and 5P. The delay of peaks later than 3P suggests that structures above the midpontine level were impaired in 6G fields. Secondary changes reflect lowered brain temperature rather than a direct effect of the gravitational field. These data indicate that the auditory system is less sensitive to hypergravic fields than is

the thermoregulatory system. Several aspects of these studies on ABRs will serve as useful background studies for experiments on vestibular far-field potentials. The studies have also pointed out the temperature sensitivity of ABRs. Finally, this lab developed techniques to measure ABRs, and to mask ABRs, which will prove useful in control experiments in vestibular studies.

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# DEVELOPMENT AND FUNCTIONAL DIFFERENTIATION OF THE VESTIBULAR SYSTEM IN NORMAL, ROTATED, AND CENTRIFUGED RATS AND MICE

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## SUMMARY

The timing of the processes of cellular mitoses and differentiation in the developing mammalian central nervous system (CNS) is a highly regulated process controlled in four dimensions. Each adult neuron is the direct result of a series of mitotic divisions by a particular cell-line in a precisely controlled positional and temporal sequence. It appears that each CNS develops in a particularly descriptive sequence reflecting its primitive embryonic derivation as well as its phylogenetic relationship. Thus, the so-called primitive neural systems appear to develop the earliest in embryonic age.

Each neural system consists of cell bodies, located in nuclei, precisely interconnected with either other nuclei or with peripheral receptors/effectors by the establishment of precisely regulated levels and patterns of synaptic connections. The patterns of synaptic contact are regulated by the singular timing sequence present during early development in which sensory, interpretive, and effector neurons must be generated within a close time frame for their mutual interaction to occur. Geometrical relationships must also be facilitated during early system generation to increase the probability of the establishment of the proper set of synaptic interconnections among the plethora of newly developing neurons. Finally, to ensure the probability of establishing a set of highly integrated and precisely patterned synaptic contacts, an excess of neurons is mitotically generated in the germinal region providing each CNS nucleus. Many of these are lost during the process of postmitotic migration from their birthplace to their final neural location. Those neurons that arrive within the proper time frame at the proper location attempt to establish a proper set of synaptic contacts and, if successful, continue their differentiation.

Stockard (1921) developed the critical period concept which in its modern expression holds that each neuron is specifically programmed to establish synaptic connections with another specifically programmed neuron and to do so only during a brief period of its early life. The failure to establish such proper synaptic contact during this critical period leads to the cell death of that neuron.

This laboratory is attempting to determine the timing patterns of cellular birth, migration, and differentiation in two phylogenetically primitive systems--proprioceptive and vestibular--in Wistar rats and two strains of mice. Included as an integral part of this study is the determination of the timing patterns and levels of cell death occurring in each element of each system during prenatal and early postnatal development.

During 1982 this laboratory has continued timed breeding in each of these species to achieve a number of staged specimens under normal (nonstimulated) conditions. The sectioning, autoradiographic processing, and serial microscopic analysis of these specimens is continuing. The accomplishments to be described are part of a continuing study and, as such, must be considered preliminary in nature. However, the data presently available do allow the following observations:

1. The data clearly indicate a limited time frame for the generation of elements of the peripheral and central integrative elements of the vestibular system.
2. This critical period of primary neuronal generation occurs at the same time that the developing embryo is undergoing implantation and maternal placentation is established. This event appears to be most susceptible to alterations in maternal cardiovascular function, a pathology to be expected under conditions of null-gravity adaptation.
3. The only nuclei of the lower brainstem (involved in proprioception) that we have sufficient data on are the second-order sensory relay nuclei gracilis, cuneatus, and the ext. cuneate and these do not appear to show a timing gradient that would reflect the normal adult topographic patterns although there does appear to be a size gradient with the larger cells (believed involved in muscle proprioception) being generated earlier than the smaller cells.
4. Cell death in the sensory regions of the vestibular membranous labyrinth is almost nonexistent (probably diffuse and missed).
5. Centrally, the onset of significant cell death in the vestibular system occurs in the superior and inferior vestibular nuclei on Embryonic Day 14, reaching a peak by Day E16 and persisting at low levels into the postnatal period. The lateral vestibular nucleus, receiving predominantly utricular input, shows a marked level (15 to 20%) of cell death on days E16 and E17, roughly 3 days after the peak generation times observed for the Maculae of the utricles, while the medial vestibular nucleus, receiving inputs from a variety of peripheral receptors, shows the lowest level of cell death through the fetal and early postnatal periods.

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## DEVELOPMENT OF REQUIREMENTS FOR INFLIGHT CONTROL AND RESEARCH CENTRIFUGE

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### SUMMARY

The initial objective of the animal research portion of this study was to determine whether the thermoregulatory responses effected by hypergravitational exposures in rats are due to the gravitational intensity (G) alone or whether they are also affected by the rotational rates and radii of the centrifuge used. Can a smaller-sized centrifuge operating at a higher speed be used instead of a larger-sized centrifuge operating at a slower speed to produce the same kind of biological effect? What minimum radius and maximum rotational speed can be used in a spaceflight centrifuge which can provide satisfactory inflight 1G control data to compare with the weightlessness-exposed group of animals? Are there differences in the biological responses in animals exposed to the same G-intensity using different rotational rates and radii over longer exposure periods even though shorter exposures may produce no differences? These are among the important questions for which answers are being sought.

A series of experiments was run in which different groups of rats were exposed to 1.41G using various combinations of rotational rates (RPM) and radii over a 30-minute period. A comparison of both the rectal temperature response (decrease) and tail temperature response (increase) between the various groups showed that essentially the same degree of change in temperature was effected in all of the groups. The RPM ranged from 22.6 to 44.0 while the radius ranged from 173 cm to 46 cm. We conclude from this study that the changes in body temperature in hyper-G-exposed rats are due solely to the G-intensity and are independent of the radius and RPM. It remains to be determined whether this will hold up for even shorter radii (less than 46 cm) and even higher RPM rates. Hence it appears that with respect to thermoregulatory responses identical responses are obtained using as small a centrifuge as 46 cm (1.5 ft.) or a much larger centrifuge (173 cm = 5.7 ft.).

These temperature studies preempted the use of the centrifuge for long exposure duration studies; these studies will be conducted in the near future. These longer duration studies will be concerned with growth rate comparisons of rats exposed to the same G-intensity but employing different radii and RPM rates as was done in the short exposure temperature studies. In addition to thermoregulatory function studies, a limited number of experiments have been completed in which simultaneous

measurements of blood constituents (glucose and corticosterone) have been performed in an attempt to see whether changes in body temperature could be associated with changes in these two constituents. Both glucose and corticosterone elevations are associated with the generalized stress response. Although preliminary results indicate an apparent correlation of the temperature response with changes in blood and corticosterone levels, the studies clearly show that the temperature response is far more sensitive and reliable as a stress index than the other two parameters.

# ROLE OF GRAVITY PERCEPTION IN THE ESTABLISHMENT OF THE DORSAL/VENTRAL AXIS OF THE AMPHIBIAN EGG

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## SUMMARY

The main goal of this research program is to understand the role gravity plays in establishing the pattern of early amphibian embryogenesis. The amphibian egg has been chosen as a model system. It offers several distinct experimental advantages: (1) it is easily obtained in large numbers, (2) it displays a distinct and reproducible dramatic response to gravity when fertilized (it "rotates" so its darkly pigmented hemisphere opposes gravity), and (3) it can be manipulated in the laboratory in several straightforward ways (e.g., it can be held in abnormal gravity orientations).

During 1982 a number of observations have been made on pattern formation in "inverted" eggs, i.e., eggs that were held upside down from the period before fertilization until the neurulation stages (by which time pattern formation is essentially completed). Remarkably, several aspects of early pattern formation (e.g., cleavage pattern, involution, etc.) were normal. That is, the external features of morphogenesis proceeded in a normal fashion. When examined by conventional histological approaches, however, it was observed that although some major cytoplasmic components shifted almost completely to accommodate gravity, not all components did. Yet the pattern of morphogenesis was surprisingly normal.

For example, large yolk platelets displayed a substantial shift, while the germ plasm (a collection of granules localized in the egg's original vegetal-facing gravity-hemisphere) does not shift at all. Those observations are being employed to develop an appreciation for the extent to which the amphibian egg cytoplasm is rigidly organized for early embryonic pattern specifications. At the present time it appears that the amphibian egg is less rigidly organized than believed by earlier embryologists. The egg is capable of tolerating a substantial amount of gravity perturbation. It is still not possible, however, to predict whether frog eggs will be able to develop normally in space (see below).

Careful examination of the cytoplasmic rearrangements that occur in normal orientation eggs and inverted eggs suggests that under normal fertilization conditions, cytoplasmic components may, in fact, rearrange according to their intrinsic buoyant densities.



It is not yet known whether inverted eggs compensate for gravity effects, since they appear to develop normally; but under natural (ground-based) conditions it appears that gravity-driven cytoplasmic rearrangements may occur. We have formulated those observations into a "density compartment" model. This model proposes that upon activation (fertilization), internal egg components stratify according to their densities.

Further experiments are being designed to test a set of predictions that this model offers. The outcome of these experiments should generate a sound basis for predicting whether amphibian eggs will be able to polarize (following fertilization) and develop normally in space.

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## EFFECTS OF WEIGHTLESSNESS AND HYPERGRAVITY ON DEVELOPMENT AND AGING OF DROSOPHILA

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### SUMMARY

The fundamental mechanisms of development and aging in Drosophila melanogaster (fruit fly) were examined under normal laboratory conditions in comparison with fruit flies housed in clinostats and centrifuges during various periods of their life cycles or exposed to weightlessness onboard Cosmos biosatellites. Investigators completed the study of the relation between the rate of oxygen consumption and lifespan in three wild strains (OR-R, D-32, Sw-C) and two mutants (w/w and w/mei-41-D5) of Drosophila. (The w/mei-41-D5 is a mutant whose embryonic tissue showed deficiency during post-replication repair.) There was an inverse correlation between median lifespan and oxygen consumption for flies having normal DNA repair. In contrast the w/mei-41-D5 showed an abnormally short lifespan, significant deficiency in mating performance, and a depressed metabolic rate, even during the first days of adult life. In contrast to a widely held opinion, these results suggested that flies, who are deficient in DNA repair, are not suitable for gerontological studies because their short lifespan does not result from accelerated aging, but from specific genetic diseases.

Morphology of worker bees exposed to weightlessness during the Space Shuttle Columbia's third flight was compared to Drosophila. This analysis was conducted in collaboration with the Electron Microscopy Laboratory at Ames and NASA's Shuttle Student Involvement Program. This preliminary investigation on the effects of near-zero gravity on the biology of social insects of the order Hymenoptera showed that the Shuttle flight did not result in morphological injury to the bees, in contrast to the wing injuries seen previously in Drosophila flown on Cosmos. The difference between the responses of flies and bees to weightlessness resulted from the bees' inability to fly in weightlessness, as shown by movies taken onboard the Shuttle. Therefore, bees were not likely to injure their wings in weightlessness. Unlike bees, the highly sophisticated flying mechanisms of Drosophila incorporate gyroscope-like halteres, and may have allowed fruit flies a certain amount of poorly controlled flight in weightlessness, resulting in injury due to impact against the walls of the housing unit.

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## COMPUTER-ASSISTED VIDEO HISTOMORPHOMETRY OF ANIMAL SPECIES

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### SUMMARY

Investigators successfully completed a method of computer analysis to measure the cross-sectional growth in the cortical bone of rats. To relieve the time-consuming and tedious point-counting of microscopic specimens, computer software was developed to digitize a bone image into a frame buffer display on a TV screen to trace tetracycline labels. The operator corrects the trace if it goes astray. Perimeter and area measurements, generated from the traced labels, are used to calculate bone formation and apposition rate. Tests with multiple bone sections, analyzed by point-counting and with a digitizing table, were within 5% of computer measurements. In addition, a statistics package calculated the mean, standard deviation, and t-statistic for each experimental parameter. This technique of image processing and analysis compared favorably with other techniques, and demonstrated very low variability of data due to the observer.

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## STRUCTURAL DEVELOPMENT AND GRAVITY

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### SUMMARY

The major thrust of this research is to investigate bone changes in a rat model which simulates the lack of weightbearing (only in the rear limbs) and the shift of body fluids into the chest and head which occur during spaceflight. Animals of various ages and strains will be used in this research; thus, the first series of studies will establish normal bone parameters in two different strains of rats throughout their lifespan. Bone formation decreases with age but this change varies from strain to strain and from bone to bone and even different sites within the same bone; thus, the first objective of this research is to define changes in bone formation rate and bone structure with age in normal rats so that realistic research goals can be established for rats of various ages and strains. A second objective of this research program deals with defining the type and extent of changes in bone structure and mineral metabolism that might occur in young rats during a routine shuttle mission (2-10 days) by using the rat model. A third objective of the program is to determine whether interpretation of data from the model is compromised by excessive amounts of physiological stress. This objective is addressed by comparing data from animals exposed to a known chronic physiological stress (4°C) with animals on the model.

The accomplishments in the first objective include processing bones from rats at 1 month, 2 months, 4 months, or 8 months after birth. The rats remaining will be 16 months of age in August. Two different strains of rats are being used; one strain attains a mature mass of about 800 grams while the other group only weighs about 400 grams at maturity.

In support of the first objective, bones have been processed for histomorphometric analysis from 1-, 2-, 4- and 8-month-old rats and will be processed from 16-month-old rats in August 1983. X-rays have been taken of the 16-month group at 1, 2, 4 and 8 months of age to determine approximate growth rates of entire long bones, vertebra, etc., and to determine time that growth in length stops. Rats are weighed three times each week to provide a growth curve of each strain of rat. These analyses allow definition of the processes occurring and the rates of bone formation at specific sites within each bone without perturbing the animals experimentally.

For the second objective, various radioisotopes that monitor cell activity and cell synthesis of certain organic substances are being given to determine the most appropriate time to label and the maximum number of isotopes that can be given to get meaningful information from a single animal. Urine and fecal samples were collected and analysis of calcium, phosphorus, and other minerals in these samples are in progress. These metabolic studies will determine whether mineral excretion is altered in the rat model with time. The studies lasted 3 weeks with collections being taken every day the first week and three times a week thereafter.

Rats cold exposed for 8 and 24 hours/day (2 rats per plastic cage with sawdust bedding) did not show a stress response. Thus, a final experiment was conducted in which the rats were individually housed in the cold in metal cages. A basal group was used at the beginning of the experiment to determine muscle mass and dynamic bone parameters. Animals (1) fed at will and (2) fed the average amount of food consumed by the rats on the model were the experiment controls. Preliminary data suggest the following:

1) Model animals ate about 17 grams of food/day the first week, about 20 grams per day during the second week, and about 22 grams per day during the third week of the experiment. Cold-stressed rats consumed about 42 grams per day during the third week while the ad libitum rats (those allowed to feed at will) ate about 26 grams per day during the third week. Interestingly, despite the differences in food intake, the cold-stressed animals gained weight at about the same rate as the group-mean-fed controls and supported animals with all groups having a mean weight of about 233 grams during the third week of the experiment. The ad lib rats weighed an average of 275 grams at the same time period. The cold-exposed rats may have been shredding, rather than eating, some of their food.

2) Thymus weight and adrenal mass, indicators of stress, changed only in cold-exposed animals.

3) The mass of the extensor digitorum longus (EDL) and gastrocnemius (G) muscles in the leg were less in the experimental than in the corresponding control groups, but greater than the basal controls. In contrast, although the cold-stressed animals also demonstrated about a 20% decrement in soleus (S) muscle mass at all time periods as compared to corresponding controls and a gain over the basal level, the "model" rats showed at least a 50% decrement in the S with the mass always being less than the basal group.

4) Bone parameters from this experiment are presently being processed.

Although not included in the actual objectives of this research, three other programs have been supported and data obtained. In a study to support the Cosmos monkey spaceflight, our results from the proximal femurs showed that bone formation was reduced in casted monkeys. Osteons, the basic units in the bone that consist of a central canal and a concentric radiation of bone cells, slowed their rate of depositing bone mineral. The percentage of osteons in the bone did not change significantly, however. The supporting trabecular surfaces in the humerus and femur were more severely retarded than the osteon, or Haversian, system which is found in bone shaft. The second study was in support of the Student Shuttle Involvement Project (SSIP). Surprisingly, model rats did not develop the systemic arthritic inflammation to the same extent as control rats. Although the mechanism for this difference is unknown, the data suggest that fluid shifts and/or unloading the sensitive paws in some way interferes with the disease process and, presumably, the immune system. The answers to such alterations in the immune system may be found in the third study. A dramatic decrease (80%) in production of alpha and beta interferon was observed in those animals exposed to simulated weightlessness as compared to control rats. These data suggest that weightlessness simulation may alter certain immunological functions in the rat.

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## RENAL FUNCTION, WATER AND ELECTROLYTE BALANCE, AND INTESTINAL TRANSPORT IN HYPOKINETIC ANIMALS

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### SUMMARY

Research in this laboratory has focused on three areas during 1982: (1) muscle composition and function during hypokinesia and hypodynamia (H/H), (2) fluid and electrolyte shifts as they impinge on cardiovascular changes, and (3) modification of the animal support system to utilize large adult rats and the laboratory mouse.

Research is concluding in the area of muscle physiology which involves the protein, DNA and RNA relationships in four hind-limb muscles: soleus, gastrocnemius, plantaris, and extensor digitorum longus (EDL). They represent the range of muscle function, from antigravity (load-bearing) to non-load bearing. The soleus responded with the greatest degree of atrophy and the most pronounced protein and RNA decrease during a 1- and 2-week period of H/H. At the same time, the EDL, a non-load bearing muscle, exhibited little or no change in these biochemical parameters. The gastrocnemius and plantaris show intermediary changes, indicative of their mixed functional roles in terms of antigravity functions. The DNA contents in all four muscles showed relatively little or no change during a 2-week support period. It was concluded that there was a decrease in muscle protein synthesis as a reflection of the protein/RNA relationship, principally in the soleus.

Studies are currently in progress to determine the mechanisms which underlie the protein/RNA changes. A Space Biology Research Associate working in this laboratory has a program of research dealing with the involvement of glucocorticoid receptor levels in muscles undergoing H/H atrophy due to unloading. Evidence of a potential role for glucocorticoids in disuse atrophy is found in reports that with H/H there is adrenal hypertrophy and increased circulating corticosterone; also, rats exposed to weightlessness showed adrenal hypertrophy. The current experiments are aimed at identifying changing levels of tissue sensitivity to glucocorticoids by assessing receptor concentrations in the four muscles identified above. Initial results showed a 400% increase in receptor levels in soleus, about 50% in the gastrocnemius and plantaris, and no change in EDL.

Having identified that diuresis and natriuresis occur only in head-down-tilted suspended antiorthostatic (AOs) subjects, but

not in orthostatic (Os) rats, this laboratory utilized the Gauer/Henry concept that AOs animals are responding to an expanded thoracic blood volume and that cardiopulmonary stretch receptors have responded to those changes in central blood volume. It was reasoned that blood pressures could reflect AOs and O positioning. Investigations were aimed at assessing cardiovascular responses of AOs and Os rats, and to a rapid head-up tilt of 70°. Direct blood pressures, i.e., mean arterial pressure (MAP), systolic, diastolic, and pulse pressures and heart rates were taken from aortic cannulations. There was a significant increase in blood pressure during Day 1 in both Os and AOs rats. In the Os rats, the MAP returned to control levels on Day 3; in contrast, the MAP remained elevated in AOs rats. This difference was chiefly the result of diastolic pressure changes. It was reasoned that the elevated blood pressures on Day 3 are a reflection of the AOs positioning and relatable to fluid and electrolyte changes which are evidenced after the 2nd day of support in AOs subjects. As anticipated, during Day 1, a rapid head-up tilt showed increases in MAP in both Os and AOs rats and also increased heart rates. However, the response was less pronounced in the AOs subjects, and on Day 3 there was a lack of MAP response. This suggests an adaptive response, probably increased vasomotor tone, as a result of AOs positioning, and the elevated fluid and electrolyte losses. Upon return to a pretilt position, blood pressures returned to the pretilt levels. These initial experiments support the previous hypothesis of significant differences between the antiorthostatic-positioned and orthostatic-positioned supported rats.

Two additional series of experiments were conducted. The support apparatus was adapted for mature adult rats weighing 450 to 500 gm. They responded in much the same manner as 180 to 200 gm animals used in previous experiments, i.e., an initial body weight loss, a loss of muscle mass (soleus > gastrocnemius = plantaris > EDL). A highly modified support system was adapted to the laboratory mouse. This is a collaborative experimental series in which the role of support H/H on interferon production is being ascertained.

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# A GROUND-BASED COMPARATIVE STUDY OF THE EFFECT OF WEIGHTLESSNESS ON THE DEVELOPMENT OF AMPHIBIAN EGGS

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## SUMMARY

Bilaterality is a basic theme of animal organization and seems to have arisen from animals of radial symmetry. This phylogeny is reiterated as the first step in the development of every bilateral animal, including man, that starts life as a radially symmetrical egg. Dating from the 19th century, hypotheses relating this change in symmetry to the action of sperm or of gravity have been debated without resolution. With the advent of space biology the means to resolve this question have become available, and, in preparation for in-space studies, a rich body of new evidence confirms that both sperm entry and gravity are involved, but "how" remains uncertain.

This laboratory has examined the dynamics of the initial rotation of the frog egg, and by the use of clinostat techniques has more nearly pinpointed the short period relative to this rotation when the action of gravity is critical. Now detailed study at the physical/chemical level is needed to understand the mechanisms by which gravity influences bilateralization.

Basic to the examination of this question is the recognition that all of evolution has been in the presence of gravity--a fact that has received little attention from biologists who have generally taken gravity as a constant and thus consider it irrelevant to questions concerning biological variation. However, gravity acts on every mass, and every organism has had to adapt to its presence, even at the initial stages of development. Moreover, its local action is not mediated through a receptor-transducer system, the typical mechanism by which organisms deal with environmental variables.

With this recognition, an expression was derived to describe the torque produced on eggs by the action of gravity on the cytoplasm which is heterogeneously distributed within the egg. It was immediately seen that all cells and defined structures in the body are subject to torque, and that this varies from  $2.5 \times 10^{-13}$  to  $8.3 \times 10^{-1}$  dyne-cm for cells ranging from 6.4  $\mu\text{m}$  to 31  $\mu\text{m}$  in diameter. Of major significance was the discovery that this torque is proportional to the fourth power of the diameter of the body.

One finds that (1) gravity imparts torque to cells; (2) since this torque is proportional to gravity, it is reduced to zero as gravitational acceleration is reduced to zero; and (3) the torque is highly sensitive to the size of the cell (structure) and the distribution of its subcellular particulates (components).

Considering that the orientation of every cell (structure) relative to its neighborhood is critical to its normal function and that gravity tends to distort this orientation, one is led to the previously unlabeled concept of positional homeostasis and to two major hypotheses:

Hypothesis 1: Energy, to the extent needed to counteract the action of gravitational force, must be expended by the cell (structure) to maintain its position dynamically or to create and maintain the structures involved in holding it in position.

Hypothesis 2: Evolution in a 1G environment has been accompanied by the elaboration of machinery for the maintenance of positional homeostasis and, energetically, this is a costly investment.

These concepts and hypotheses find experimental support in the apparent decrease in glucose utilization (58% less) by tissue culture in spaceflight (Montgomery, et al., 1977). They are not contradicted by evidence that Brownian movement dominates over the action of gravity on bacterial cells, diameter  $<5\text{ }\mu\text{m}$  (Pollard, 1977).

Various secondary hypotheses derive from the above. Interesting among these is that Brownian movement and gravity define the lower and upper limits of cell size for cells subject to the principle of positional homeostasis. One of the recent tasks on this project has been to examine cell size in this context. It has been found that few metazoan cells are less than  $10\text{ }\mu\text{m}$  or greater than  $40\text{ }\mu\text{m}$  in diameter and that the functions attributed to them can be related to their location in this size range.

Other secondary hypotheses concern the mechanisms which protect cells from displacement by the action of gravity. Examination of these hypotheses has led to interesting new interpretations of cell and tissue structural organization.

Examining these hypotheses for physiological significance leads to the prediction of marked metabolic changes associated with exposure to either hypo- or hypergravity, and the reexamination of the sparse relevant literature reveals that indeed there is strong evidence of metabolic changes that can be attributed to the influence of gravity on the positional homeostasis of cells and organs or their parts. The next task is to conduct experiments to confirm this relationship.

This laboratory is also examining the influence of gravity on cells resulting from its generation of other quantities: tension, compression, shear, strain, and simple positional displacement. The calculations and evidence all point in a direction indicating that gravity may be one of the most significant environmental variables influencing the structure and function of organisms and that exposure to changes in the value of this variable will lead to fundamental structural and physiological changes, many of which may be expressed on a time line which has only now begun to be accessible to manipulation.

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# HYPER-GRAVITATIONAL EFFECTS ON METABOLISM AND THERMOREGULATION

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## SUMMARY

The main objectives of this research are to determine how changes in gravitational fields affect the ability of mammalian animals to maintain their body temperature and blood sugar level. Both show rapid and substantial departures from the levels observed in normal-gravity-maintained animals when subjected to hypergravity conditions. The deviations observed have been closely related to the gravitational intensity (G) which is imposed on the animal and the duration of exposure.

The blood sugar deviations have been investigated by studying processes that control the rate at which sugar leaves the blood (utilization rates) and the rate at which it enters the blood (formation rates). Both neural (sympathetic nervous system) and hormonal (insulin, glucagon, catecholamines, corticosterone) systems are being studied in rats under hypergravitational conditions.

In a similar manner, body temperature regulation is being studied by determining the rate at which animals lose heat as well as generate it under hypergravity. The balance between the two rates determines the body temperature. Both neural and hormonal mechanisms which regulate heat loss and heat production are under investigation. Contraction and dilation of blood vessels which help regulate the rate of heat loss are being monitored through the use of surface temperature probes while heat production is being determined through measurements of oxygen consumption rates. The basic studies are directed toward developing a better understanding of how gravity and gravitational changes affect systems that help maintain the internal environment of the animal at a balanced state in spite of marked changes in the external environment.

Among the significant accomplishments during 1982 have been the completion of studies to quantify the deep body (rectal) temperature decrease and surface (tail) temperature increase responses in hypergravity-exposed rats. Incremental changes of both have been found between 1.0G and 1.5 to 1.8G. Higher G intensities produced no further decrease in rectal temperature or increase in tail temperature. The tail temperature response which actually decreased with higher G loads indicates that mechanisms to constrict the blood vessels of the tail (sympathetic nervous system) and decrease the rate of heat loss become activated at the higher G intensities but not at the lower

intensities. There appears to be a threshold for activating the sympathetic system. Investigations are currently underway to study this interesting response further.

The results obtained lend further support to the view that the rectal and tail temperature response system is one of the most reliable and practical means of monitoring and quantifying the stress of hypergravity exposures in rats. It is a sensitive system with as little 0.1G difference effecting a different rectal and tail temperature change. Another interesting finding is that exposing rats to hypergravity in either a head-downward or head-upward position (tilt angle = 29 degrees) had no significant effect on the rectal or tail temperature response. These results indicate that thermoregulation under hypergravity conditions is not affected by changes in body position but is sensitive to the field intensity.

Accomplishments dealing with the metabolic aspects (glucose balance) in hypergravity-exposed rats include the completion of blood lactate and glycerol response patterns for periods ranging up to 24 hours of exposure. Hormonal patterns have also been established for insulin, glucagon, and catecholamines. These hormones are major determinants in regulating the utilization of blood sugar as well as its production by the liver. The latter process appears to be the primary regulator of blood sugar elevation during the initial stages of hypergravitational exposures. What role this process has in sustaining the evaluation of blood sugar over more extended exposures remains to be elucidated. During 1982, techniques have been developed to prepare viable liver cells from hypergravity-exposed rats for testing the state of the liver tissue to form new sugar from noncarbohydrate sources, i.e., amino acids, and from the breakdown of fat (glycerol) and muscle glycogen (lactate). By using this isolated cell system preparation, many types of studies which cannot be conducted in the intact, living animal can be performed under well-defined conditions. In developing the procedure, an elaborate system for perfusing the liver tissue under carefully controlled conditions was assembled and successfully placed into operation. The new procedure will permit study of the effects of individual hormones such as glucagon on sugar formation capacity by the liver cells of hyper-G stressed rats separated from the effects of other hormones which are normally present in the intact animal.

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# PRIMATE HEMODYNAMICS AND METABOLISM UNDER CONDITIONS OF WEIGHTLESSNESS

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## SUMMARY

A major accomplishment during 1982 was the preparation of a formal Flight Verification Analysis of a Primate Metabolic System in collaboration with the McDonnell Douglas Astronautics Company, Huntington Beach, California. The report defines an engineering configuration of a system capable of maintaining a 10 to 14 kg adult macaque in comfortable seated restraint in the mid-deck of the Shuttle during flights of up to 7 days' duration, or longer. The design is based on several years of development and testing of a monkey restraint habitat in this laboratory and in NASA flight payload simulations.

The system provides appropriate ventilation, food and water, and excreta collection, as well as continuous physiological monitoring of the animal during the period of the flight. The design emphasizes the use of components already developed, and in some instances already flown, by NASA in other flight programs in order to minimize costs.

A plan is proposed to construct an Engineering Development Unit to test the system concept with a live animal in a NASA Mid-Deck simulator for several days before a decision is made to proceed with fabrication of a Flight Unit. It is further proposed that the Flight Unit be tested in a short duration, 2- to 3-day Shuttle flight, followed by a longer, 5- to 7-day Shuttle flight using monkeys with no surgical preparation. This plan provides conservative examination of the potential for use of large primates in space life sciences research in a cost-effective manner.

During 1982 substantial progress was also made in developing a battery of radioimmunoassay procedures for assessment of the status of thyroid function in macaques. It has been known for many years that the metabolic rate of the body is regulated by thyroid hormones circulating in the blood. More recently, it has become evident that there is a complex feedback relationship between metabolic rate, the blood thyroid hormone levels, and the activities of the pituitary and thyroid glands. Thus, in evaluating changes in metabolic rate it is necessary to define the correlative changes in thyroid system activity.

The seven principal blood parameters evaluated were: thyroid

stimulating hormone (TSH) produced by the pituitary gland, thyroxine (T4) produced by the thyroid gland, triiodothyronine (T3) produced in part by the thyroid gland and in part from T4 in the body tissues, and a T3 isomer (rT3) produced in the body tissues. TSH stimulates the thyroid gland to produce T4 and T3. In turn, both T4 and T3 stimulate oxygen consumption by body cells, T3 being six times as potent as T4, while rT3 has no effect on oxygen consumption. T4 and T3 are largely bound to serum proteins and, because only the free form of these hormones is physiologically active, it is also necessary to measure the serum concentration of thyroxine-binding globulin (TBG), of free T4 (FT4), and of free T3 (FT3).

The close phylogenetic relationship of macaques to man permits the use of radioimmunoassay procedures developed for human blood, and commercially available kits are available for all the thyroid parameters noted above with one important exception--FT3. Accordingly, in 1982 the project was successful in developing a radioimmunoassay procedure for FT3 in small volumes of macaque serum. A protocol was evolved for measuring the seven principal thyroid hormone parameters in 1.5 ml of macaque serum, so that sequential evaluation of thyroid function status in a particular animal is practically possible.

In one 1982 study, measurements were made of five of the seven parameters (all but TBG and FT3) in blood samples collected 3 weeks apart from 17 of our colony (adult male, Macaca nemestrina). Good test-retest reliability was demonstrated. In another study, it was demonstrated in three monkeys that TSH, T4, T3, and FT4 serum levels were unaffected by continuous seated restraint for 8 days in our Environmental Physiology Laboratory (EPL) monkey pod.

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## GRAVITY, BODY MASS AND COMPOSITION, AND METABOLIC RATE

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### SUMMARY

It is common knowledge that the colder the surroundings in which an animal finds itself, the greater is the metabolic energy expenditure rate the animal requires to maintain body temperature. Less appreciated is the fact that as the surrounding temperatures approach and exceed body temperature, a greater metabolic energy expenditure by the animal is likewise required to maintain body temperature. Thus, there exists a region of surrounding temperatures in which the metabolic rate of an animal is at a minimum. This temperature range is known as the zone of thermoneutrality. It also follows that if comparative studies of metabolic rate among animals are made, care must be taken to standardize the ambient temperature conditions.

Under this grant, a four-species model--hamster, rat, guinea pig, and rabbit--has been developed to study the scaling of metabolic rate on body size among mammals. Although previous investigators have examined the thermoneutrality zone of three of these species, the work has largely been done on young, immature animals which generally have a higher metabolic rate than mature animals. During 1982, a series of measurements of metabolic rate by direct calorimetry at different ambient temperatures from 20°C to 35°C were carried out in six mature animals of each of the four species in order to define the thermoneutrality zone. Results show that the metabolic rate remained within 10% of the minimum value in the temperature range 27 to 31°C for hamsters, 24 to 30°C for rats, 26 to 33°C for guinea pigs, and 25 to 35°C for rabbits. Hence,  $28 \pm 1^\circ\text{C}$  represents an optimal temperature for all four species to pursue comparative metabolic rate studies, and probably represents the most comfortable ambient temperature for these animals.

Measurements have also been made of metabolic rate in the four-species model during chronic centrifugation for 6 weeks at 2.0G. Although the experiment is not yet completed, preliminary results indicate that the metabolic rate of hamsters with a mean body mass of 0.11 kg was increased by 6% after 2 weeks at 2.0G, that of 0.50-kg rats by 16%, that of 0.70-kg guinea pigs by 11%, and that of 3.3-kg rabbits by 36%. Because the metabolic rate of the larger animals was increased proportionally more than that of the smaller animals, it may be concluded that gravitational loading definitely influences the scaling of metabolic rate on body mass such that at 2.0G the scaling is probably close to the 0.80 power rather than to the classic 0.75 power seen under

normal gravity conditions. The implication is strong that the scaling under conditions of weightlessness will be significantly less than the 0.75 power.

The development of a method for estimating skeletal muscle mass of mature animals of our four-species model from the body creatine content was completed. The appropriate conversion factors were derived experimentally by analysis of creatine content of fat-free skeletal muscle samples. For hamsters, body skeletal muscle mass in grams is given by multiplying body creatine content in grams by 291, for rats multiplying by 227, for guinea pigs by 237, and for rabbits by 217. It was also shown that the four species display consistently different degrees of muscularity, the skeletal muscle content of the fat-free body mass ranging from 43% in hamsters to 53% in rabbits.

A collaborative study with Emily Morey-Holton of the NASA Ames Research Center on the effects of 14 days of hindquarter unloading on body composition of 80-day-old and 270-day-old rats was completed in 1982. The results were compared with similar data obtained earlier on rats from Cosmos 1129 after 18.5 days of weightlessness. It was concluded that hindquarter unloading mimics the effects of weightlessness in some respects, such as the skeletal changes in young, growing animals, but that important differences also exist. Therefore, results from suspended animal experiments should be interpreted with caution.

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# EFFECT OF DECREASED GRAVITY ON CIRCULATION IN THE RAT

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## SUMMARY

Gravity has a profound effect on some processes in mammals. The effect of gravity on cardiovascular systems is especially great, (e.g., blood pressure in the small blood vessels (arteries or veins) in the legs of a standing man is increased to 120 to 220 mm Hg instead of the much smaller values observed when man is in a horizontal position, as in bed). Thanks to many cardiovascular (mostly reflex) mechanisms and many adaptations developed during millions of years of biological history, blood does not pool in the legs of a standing man but returns toward the heart. In a weightless state the blood returns easily toward the heart because the high hydrostatic pressure in the lower extremities disappears and the mentioned circulatory mechanisms are not needed or used. Circulatory (orthostatic) intolerance and a decreased work capacity are observed after return to Earth, and these changes (shifts in blood distribution, overloading of the atria of the heart because of the movement of blood toward the chest, neurohumoral stimulation leading to excessive water loss, and a profound blood volume loss concomitant with other circulatory changes) persist postflight and require readaptation of the circulatory system to 1G.

In order to better understand circulatory changes induced by weightlessness and to identify physiological mechanisms responsible for these changes, an animal model was developed that mimics circulatory effects of weightlessness. The cardiovascular measurements were performed in control experiments on Earth in unrestrained, unanesthetized rats; and in the same animals in head-down hypokinetic conditions; and during readaptation of the same animals to free activity. The aortas and right atria of the animals were permanently cannulated 15 days before experiments and an electromagnetic flowmeter was implanted around each animal's aorta. Arterial and central venous blood pressures, heart rate, cardiac output, and other cardiovascular parameters were measured after 15 days (full recovery of the animals). The animals were undisturbed and seemed to be unaware that any work was being done on them. Seven-day experiments (corresponding to 7-day Space Shuttle/Space Lab experiments) have shown that the hypokinetic rat initially had an increased right heart atrial pressure (congestion of blood in the chest), a decreased arterial pressure, an increased stroke volume, and an increased cardiac output. After 2 days these parameters returned to normal. After release from the harness the rats showed a decreased ability to

"work" (exercise on a treadmill), and their heart rate was very much elevated while their cardiac output was significantly decreased. In order to study the effect of stress (induced by harness and head-down position) on cardiac output increase, levels of "stress hormones" were measured in the blood of the antiorthostatic rats.

On the basis of the results it was concluded that stress induced during early exposure to antiorthostatic hypokinesia is one of the reasons for the reported increased cardiac output. However, the animals adapted to the new situation quickly. After a few days of exposure the increased plasma hormonal levels returned to control values.

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## BONE CELL KINETICS OF SIMULATED WEIGHTLESSNESS

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### SUMMARY

This is a new project. Studies of rat weight-bearing bones indicate that the osteopenia of weightlessness is due to inhibited bone formation with no appreciable effect on resorption. Cosmos 1129 results suggest suppression of bone formation may be more generalized because preosteoblast differentiation is depressed in the periodontal ligament (PDL) of rat molars in nonweightlessness maxilla. The hypothesis to be tested is that weightlessness is associated with a generalized (systemic) suppression of osteoblast precursor cell proliferation and differentiation, resulting in marked inhibition or arrest of bone formation. The number of preosteoblasts will be determined, utilizing the nuclear morphometric assay, in the diaphysis and metaphysis of ulna bones from Cosmos 1129 rats (18.5 days of weightlessness). Results will be compared with similar data previously reported for maxillary molar PDL. Bone precursor cell proliferation osteoblast activity and osteoclast activity will be assessed autoradiographically by using  $^3\text{H}$ -thymidine, -proline, and -leucine in the maxilla and long bones of rats subjected to simulated weightlessness (head-down support). A double labeling  $^3\text{H}/^{14}\text{C}$  method will be developed for simultaneously assessing bone cell activities with two labeled compounds. A suitable double marker technique for labeling animals immediately after return from space would be advantageous in future missions for studying reinitiation of normal bone modeling/remodeling.

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## MAMMALIAN GRAVITY RECEPTORS: STRUCTURE AND METABOLISM

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### SUMMARY

The long-term goal of this research is to shed light on mechanisms underlying calcium metabolism by gravity receptors. This will in turn lead to a better understanding of the otoconial complexes they contain.

Otoconial complexes consist of tiny, microscopic-size crystals of a polymorph of calcium carbonate (calcite in mammals) together with a small complement of organic material. Organic material also binds the crystals together and to the underlying receptor area. The crystals are called "otoconia" (literally, "ear dust") and the organic substance connecting them to the receptor sites in the saccule and the utricle of the inner ear is the otoconial membrane.

Organic material is continuous from the otolithic membrane to the inside of the crystals, but it is unclear whether the two materials, inside and outside the otoconia, are identical in chemical composition. Embryologically, the organic material is incorporated into the crystals before a well-defined otoconial membrane is evident.

It has been accepted for many years that the otoconia, by virtue of the mineral they contain, add mass to the otoconial membrane, making the sensory cells of the receptors more sensitive to linear acceleration. It is thought that an inertial force acting upon the more dense crystals changes their position relative to the sensory cells as acceleration is applied. Research on this project, however, has begun to cast doubt on this simplistic explanation of otoconial function, as the following points should help illustrate:

1. Otoconial complexes take up and release calcium ions within relatively short periods of time. Uptake is nearly complete within 1 hour, which compares favorably with uptake in bone mineral; while release is nearly complete within 4 days, which is much faster than in bone. Thus, otoconial complexes are dynamic, not static, structures. The speed of uptake and release is too fast to be accounted for on the basis of new calcite formation by ordinary mechanisms of crystal growth, implicating the organic material of the complexes in short-term ion uptake and release.
2. Fetal studies in the rat have shown that the organic and



mineral phases are closely aligned during otoconial crystal growth and that multiple seeding sites are present. Coalescence of the crystallites and organic material into adult crystal form takes place later. These findings suggest that the organic material serves as seeding material for the crystallites and guides their further growth in a relatively strict pattern; it may also be responsible for limiting their growth.

3. Micro-disc gel electrophoresis studies of the proteins of the complexes revealed that a protein with the molecular weight of 16,000 to 17,000 daltons is present and is extractable with EDTA. Calmodulin, a calcium-regulating protein present in both plant and animal cells, has a molecular weight in this same range (15,000 to 19,000 daltons, depending upon experimental conditions) and is similarly EDTA extractable. It is possible that calmodulin is a component of otoconial complexes even though the latter are extracellular constituents of gravity receptors. The surrounding fluid in the compartment in which the otoconia function is high in potassium ions and low in sodium ions, mimicking intracellular ionic conditions.

4. Ultrahigh resolution studies of the otoconia demonstrated that the otoconia are not single crystal in nature, as was previously thought. Instead, they are highly ordered composites of organic and inorganic materials, with the inorganic material consisting of crystallites that are not comparable in appearance to those of pure calcite. This again suggests that the mineral is seeded and ordered by the organic phase. Moreover, the morphology of the crystallites is not symmetrical. This lack of symmetry as well as the ordering of the crystallites in layers strongly suggests that the otoconia are piezoelectric. Thus, the crystals may function to alter the electric field around them during linear acceleration and, if so, need not move at all over the underlying receptor area to stimulate the sensory cells. It is also possible that the organic phase is a piezoelectric, or semiconducting material, contributing to the piezoelectric effect or being solely responsible for it. It is strongly suspected, for example, that it is the organic component in bone mineral that is responsible for the piezoelectric effect in that biomineralized material.

The short-term goal of this study is to carry out amino acid analysis of the organic phase of the otoconial complexes. This project was chosen as the first goal, because a body of literature already exists concerning the amino acid composition of other related substances, namely, calcite-containing shells and otoliths of fish. Fish otoliths consist largely of a different polymorph of calcium carbonate, aragonite, but are homologous with the otoconia of other vertebrate gravity receptors. Additionally, we are dealing with a very small mass. A pair of saccular otoconial complexes weighs about 9.5 micrograms while a pair of utricular complexes weighs about 14.5

micrograms. This includes the mineral! It seemed more rewarding to attempt amino acid analysis first and to accomplish protein separation and amino acid analysis of each protein later. This is because analysis of each protein represents a step into a more difficult dimension quantitatively. Moreover, methods for amino acid analysis would have been perfected and the size of the sample necessary for such analysis would have been determined prior to this step. Sugar analysis will also be required, because some sugars are important in calcium binding in other systems. Again, the amounts of material required for sugar analysis can be estimated based upon the amino acid analyses.

Because of the level of sensitivity required, high pressure liquid chromatography (HPLC) was selected for this phase of work, and postreaction with a chemical (ortho-phthalaldehyde, OPA) that makes the amino acids fluoresce has been utilized. A fluorescence detector then determines the amount of fluorescence given off by each amino acid component. The method is sensitive in the low picomolar range (10 or fewer picomoles for each amino acid).

The project's HPLC equipment was put in place fall 1982. Achievements since that time are as follows:

1. Organic material of otoconial complexes can be analyzed by HPLC in spite of the small mass, using a relatively small sample size. This is, perhaps, the most significant achievement.
2. This research has shown that quantitative and qualitative amino acid analysis is possible using a sample pooled from eight animals. Moreover, this sample apparently could be halved.
3. Amino acid profiles of the organic phase of the otoconia have been produced and procedures are under refinement.
4. Subsequent studies of the organic material utilizing alkaline hydrolysis for detection of gamma-carboxyglutamate, an amino acid found in other calcium-sequestering systems such as bone and thought to be important in inhibition of mineral deposition, showed the amino acid to be present in otoconial complexes. (Gamma-carboxyglutamate does not survive acid hydrolysis.) This work also demonstrated that hydroxyproline was present, making the otoconial complexes more comparable to otoliths than to shells in composition.

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## GROUND-BASED MODELS FOR HYPOKINESIA

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### SUMMARY

In order to understand the effects of spaceflight on the weight-bearing and non-weight-bearing skeletons, an animal model system that at Earth's gravity produces changes in bone structure similar to those recorded after exposure to 0-G conditions was employed.

The effect of simulated spaceflight on the growth and maturation of the non-weight-bearing skeletons was compared in young (41-day) and old (1-year) rats. The animals were supported in a head-down tilt mode for 15 days, after which the lower jaws were saved and prepared for analyses of the distributions of calcium, phosphorus, and hydroxyproline (= bone collagen matrix). The jaws were subsequently ground into 40  $\mu\text{m}$  particles and these were separated into four fractions according to their specific gravity. The fractions represent a maturation gradient for bone mineral and matrix, such that the bone packets with specific gravity of 1.3 to 1.7 are very immature, and those with a specific gravity of 2.2 to 2.8 are the most mature. The animals also had received injections of tetracycline to mark the growing surfaces of their lower incisors and supporting jaw bone.

At either age, the support system failed to alter total jaw calcium, phosphorus, and hydroxyproline concentrations, or their profiles within the density gradient system in both the younger and older rats. The rates of bone growth and growth of dentin (16 to 17  $\mu\text{m}/\text{day}$ ) in the incisors remained at control levels.

The results of this study did not mirror the effect of actual spaceflight on the maturation of mineral and matrix moieties in the rat jaws. For the supported rat model to produce changes which faithfully mimic all the effects of spaceflight in rats, the skeletal response should include evidence of a delay in the maturation of bone mineral and matrix moieties. This would be seen as a shift in the concentrations of calcium, phosphorus, and hydroxyproline toward the lower specific gravity fractions.

How should one try to account for the inability of this land-based rat model to produce spaceflight-specific effects on mandibular bone maturation? The most appropriate answer to this question would seem to be that the head-down tilt model does not interfere with the continued normal functioning of the jaw's

antigravity muscles. Unlike the situation during spaceflight, the land-based experiments do not seem to moderate these normal biomechanical-structural interrelationships which are so essential to the normal pace of bone growth and remodeling.

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## EFFECTS OF MUSCLE ATROPHY ON MOTOR CONTROL

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### SUMMARY

As a consequence of U.S. and Soviet space program experiences, it is apparent that weightlessness reduces both the cardiovascular and muscular capabilities of astronauts and cosmonauts. Of these post-flight-observed weightlessness-induced responses, this project has focused on (1) the effects on muscle, and (2) the implications of these changes for the control of movement. While numerous investigators have noted the loss in whole-muscle weight and functional capability that accompanies weightlessness, little attention has been directed toward the effects upon the constituent units of muscle. Since muscle does not comprise homogeneous units, it is unlikely that whole-muscle changes represent uniform effects experienced by all the units. To extrapolate these effects from one muscle to another and to elucidate the mechanisms involved in atrophy, it is necessary to characterize the differential effects at the unit level.

Muscle represents but one component of the system responsible for movement production, the others being a neural element to command the movement and a sensory element(s) to provide information to the nervous system on the execution of the movement. Accumulating evidence suggests that should the integrity of one component be altered the others will be similarly influenced. Thus, the second objective of this project addresses the effect of muscle atrophy on these neural and sensory elements.

The experimental paradigm involves a suspension model which has been demonstrated to induce changes that parallel the responses of space-flight-subjected animals. In this protocol, the hindlimbs of rats are supported above the ground by a harness for up to 16 weeks. The animals are able to move about the cage by using their forelimbs. Following the support, measurements are made on some of the muscles and associated neural and sensory elements which contribute to movement of the foot.

The intended strategy for the research project was to quantify the extent of support-induced atrophy before beginning an analysis of the differential effect of the atrophy on the muscle units. This was to be accomplished by comparing the whole-muscle physiologic status (e.g., speed and force of muscle contraction, fatigability) of control to supported animals. However, after several experiments it became apparent that the neuromuscular connection in the experimental hindlimb muscle (medial gastrocnemius) was less than anticipated prior to support. For example, a standardized fatigue test produced more substantial

reductions in force and neural input (EMG; 79 and 66%, respectively) than we expected based on theoretical calculations (62 and 23%, respectively). The evidence suggested an impairment of the process that couples the muscle-directed neural input to the force produced by the muscle.

As a result of this finding, the initial phase of the project has been redirected to examine the fatigue profiles of muscles in exercise-deprived (small cage-reared), exercise-exposed (near-free environment-reared), and wild rats. The working hypothesis is that the cage-reared rat is inappropriate for the study of the weightless state whereas a rat of the same species reared in a near-free environment is as suitable as a wild rat. Upon conclusion of these experiments, the investigators will return to the initial goals of the project with the acceptable animal model.

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# INFLUENCE OF SUSPENSION-HYPOKINESIS ON SKELETAL MUSCLE

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## SUMMARY

Upon return from space, both humans and experimental animals exhibit a number of changes, particularly in skeletal muscle, which shows marked atrophy and altered functional and bioenergetic characteristics. The goal of this project is to identify the physiological, histochemical, and biochemical parameters of skeletal muscle which are most sensitive to zero gravity and best characterize the influence of zero gravity on muscle.

To accomplish these goals, this investigation is currently evaluating the Morey-Holton rat model for weightlessness and has characterized rats supported for up to 4 weeks and rats that have recovered for 1 week after support for 2 weeks.

The most significant accomplishment during 1982 has been the demonstration that the Morey-Holton model evokes speeding of the soleus, an antigravity muscle in the rat. Speeding refers to a decline in times required for the muscle to contract (contraction time) and relax (one-half relaxation time). The histochemical and biochemical investigations of the soleus have also provided a possible explanation for this soleus speeding.

The soleus is normally composed of from 70 to 90% Type I (slow-twitch) muscle fibers; the remaining fibers are Type II (fast-twitch). Measuring the relative amounts of Type I and II myosin in the soleus, rat suspension was found to cause a significant decline in Type I myosin without changing the Type II component. In agreement, the histochemical investigation, which used ATP-ase staining to identify Type I and II fibers in the soleus, found a change in the normal distribution of fiber types during rat suspension from the normal Type I predominance toward a greater percentage of Type II fibers.

The other histochemical data indicate that rat suspension elicits changes in the soleus muscle that are characteristic of denervation. These changes include esterase positive fibers, which are indicative of increased enzymatic activity, and pale centers or moth-eaten fibers on nicotinamide-adenine-dinucleotide phosphate staining, which indicate irregular mitochondrial distribution.

Recovery from rat suspension has been studied by supporting rats for 2 weeks and allowing recovery for 1 week. The soleus muscles



from these rats displayed a return toward control values in their contractile parameters and their Type I myosin concentration. In contrast, their histopathic changes characteristic of denervation persisted after the 1 week recovery period.

Comparing this project's data from suspended rats with that of space-traveled rats indicates that the Morey-Holton support model is worthy of consideration as a weightlessness model. Although only limited data from space-traveled rats is available for direct comparison with these results, similarities between the data do exist. These similarities include decreased muscle function and speeding of the soleus.

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## SKELETAL MUSCLE METABOLISM IN HYPOKINETIC RATS

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### SUMMARY

Weightlessness abolishes weight-bearing requirements for skeletal muscles in the legs of animals. In both astronauts and rats, this condition leads to loss of muscle mass; i.e., atrophy of the muscle ensues. Although it is known that this loss of muscle can be attributed to the disappearance of protein, the principal component of muscle, little is known about the causes for this loss and whether there are attendant biochemical alterations as well. Therefore, this project is using a suspension hypokinesia model in rats, which induces leg muscle atrophy, to understand better the biochemical adaptations of muscle to non-weight-bearing conditions. Furthermore, using this model, this lab hopes to develop a means for preventing the marked atrophy of certain leg muscles while maintaining normal muscle function.

A suspension-hypokinesia model has been used for studying the effects of reduced weight-bearing on the metabolism of skeletal muscle in the rat hindlimb. The rats are supported from the base of their tails to make the hindlimbs non-weight-bearing. Two different types of control animals are used for comparison with the experimental hypokinetic (supported non-weight-bearing) animals. One group is kept normally with one animal per cage (nonsupported). The other control group has their tails attached to the support apparatus but are permitted to walk on their hindlimbs so they are weight-bearing.

Research during 1982 has focused on two main questions. The first considered what other alterations in the biochemistry of the muscle might accompany the loss of muscle protein. This lab showed previously that the formation of protein decreases and the destruction of protein increases in certain leg muscles of the hypokinetic rats. This response leads to a greater availability in the muscle of amino acids, which are the basic units of protein. Therefore, the study of the fates of these excess amino acids in muscle was an important consideration. Two of these amino acids, alanine and glutamine, are of particular import because they provide a vehicle for removing from the muscle nitrogenous waste which is generated by the destruction of other amino acids.

Since soleus muscle undergoing protein loss in hypokinesia is likely to have excess nitrogen waste, a marked increase in the output of alanine and glutamine was expected. Instead, alanine output was unchanged and the output of glutamine was diminished

markedly. Normally the output of glutamine in muscle is controlled by glucocorticoid hormones produced in the adrenal glands. However, even after removal of these glands from control and hypokinetic animals a large difference was still observed. Hence, in muscle of the hypokinetic animals there is an impaired ability to manufacture glutamine. This observation is important because it suggests that such muscles will have difficulty removing nitrogen waste materials, which can in turn lead to impairment of certain aspects of muscle function.

The second question addressed was whether there is a way to prevent the marked atrophy of the soleus muscle and its attendant lost capacity for producing glutamine. It is well-known that constant passive stretch of a muscle can increase muscle mass; i.e., cause hypertrophy. Therefore, this lab tested whether passive stretch of one soleus muscle in the hypokinetic rat would show a diminution of the atrophy response. The results were most encouraging! The soleus muscle in the leg stretched passively during 6 days of hypokinesia showed not only no loss of mass but amazingly increased in size to a greater extent than did this muscle in normal rats. Furthermore, the passively stretched muscle seemed to show a more normal capacity to produce glutamine. Hence in hypokinesia, simple passive stretching seems to maintain muscle size and perhaps even normal muscle function. Such results suggest that passive stretch may be a useful approach for preventing muscle atrophy and loss of muscle function in astronauts.

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# EFFECTS OF SIMULATED WEIGHTLESSNESS ON MEIOSIS, FERTILIZATION, AND EARLY DEVELOPMENT OF MICE

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## SUMMARY

This is a new project. The long-range goal of this research effort is to examine the effects of one aspect of spaceflight, specifically, an altered gravitational field, on critical stages of reproduction in animals. The aspects which will be examined over the next 3 years include (1) the reduction divisions involving the female germ cells' genetic information that occur during the process of meiosis; (2) the fertilization process wherein the sperm enters the egg and sets the stage for embryonic development; and (3) early embryonic development up to the eight-cell stage.

The laboratory mouse is used as a model system since the cellular biology and genetics of the mouse are the best understood of possible animal models. Altered gravitational conditions are achieved using a clinostat, an instrument that simulates a zero gravitational state for organisms, or as in the present case, for cells or groups of cells in an in vitro culture system. All experiments utilize in vitro culture conditions in order to separate direct effects on the cells being studied from indirect effects that altered gravitational states could have via maternal factors. It is anticipated that these studies will provide important new information on the effects of altered gravitational fields on basic biological processes such as cell division, proper segregation of the chromosomes that carry the genetic information, and recognition of one cell by another during fertilization and early embryonic development. Such information will be extremely useful in predicting the effects of both long- and short-term spaceflight on animal reproduction.

In the few months since this project was initiated, the following have been accomplished:

1. Adaption of Standard Tissue Culture Conditions for Use in the Clinostat: In this lab's standard laboratory conditions, oocytes are recovered under sterile conditions from the ovaries of mature mice and placed in a petri dish with the appropriate medium to support the metabolic functioning of the cells during the reduction (meiotic) division. The cultures are loosely covered and placed in an incubator at 37° C and 5% CO<sub>2</sub> in air for 16 hours. It was necessary to alter this procedure so that the cultures might be attached to the clinostat and rotated while still permitting gas exchange and equilibration with the culture. This required assessing several different types of culture

dishes, various volumes of media, and various overlaying substances to prevent leakage. The investigators believe that they have found an appropriate culture system using Falcon No. 3072 microtitre plates, 0.4 ml of McCoy's 5a culture medium, and a 20-lambda overlay of gas-equilibrated paraffin oil. Under these conditions, the oocytes can progress to the second meiotic metaphase, which is the endpoint routinely achieved under our standard conditions.

2. Construction of a Clinostat for Use in Culturing Mammalian Oocytes: The clinostat constructed is modeled after that developed by J. Tremor and K. Souza for use in their studies on amphibian eggs and development (Space Life Sciences 3: 179-191, 1972.) The single greatest difference is that, as mentioned above, this lab's studies require an environment of 5% CO<sub>2</sub> in air and a humidified ambient temperature of 37° C. This has been achieved by constructing a clinostat that will fit into a Forma Model No. 3325 incubator in which temperature, gaseous environment, and humidity can be regulated. The initial construction uses a motor that produces rotation of 1 revolution per minute of the culture dish containing the eggs. Additional motors, with a range of rotational speeds, will now be examined. The clinostat is designed to permit easy exchange of motors, to minimize vibration of the rotating shaft, and to permit rotation of the cultures in horizontal and vertical planes.

These studies are currently focusing on determining the effects of various rotation conditions on the meiotic process and will include observations on cellular morphology.



## PLANT PROJECTS





## LOCALIZE AND IDENTIFY GRAVITY SENSING MECHANISMS OF PLANTS

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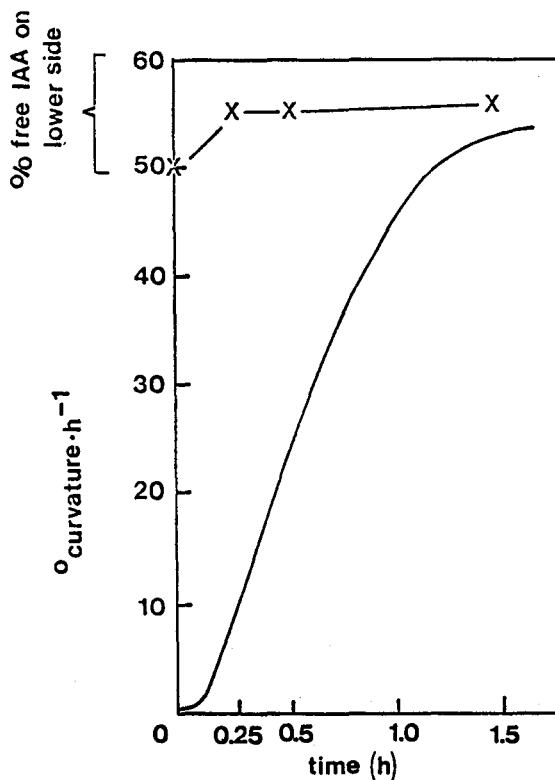
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### SUMMARY

Plant stems grow up and plant roots grow down. If the plant is displaced from its vertical orientation to the earth's surface, it responds by growing back into a vertical position. The plant accomplishes this response to gravity by altering the relative amounts of growth hormone, indole-3-acetic acid (IAA), on the upper and lower sides of the stem. How the plant alters IAA concentration in the tissue and how quickly this asymmetry is accomplished is unknown.

This laboratory, in cooperation with that of Peter Kaufman, studied the length of time required for the plant to begin its growth response to gravity and the length of time required for a change in the relative amounts of IAA in the upper and lower sides of the stem. The results of this study are shown in the figure below:



As can be seen, from the solid line, the plant begins curving back into a vertical position in about 5 to 10 minutes after being placed in a horizontal position and reaches its maximum rate of straightening in something more than 1 hour. In 1.5 hours it is straightening itself at a rate of  $50^{\circ}$  per hour--almost  $1^{\circ}$  per minute. The change in distribution of the hormone, shown by the line with Xs, is far more rapid in that in 0.25 hours, 55% of the IAA is on the lower, more rapidly growing, side of the stem. Clearly, the maximum change in hormone concentration precedes the attainment of the maximum rate of straightening. Thus, the change in hormone concentration could be the cause for the change in growth rate. However, other factors must also be involved since the growth response and the change in hormone concentration do not coincide exactly in time.

The above-described studies are continuing and are being extended to measure other forms of the growth hormone--IAA forms in which the IAA is conjugated to a sugar. From these studies it is expected to learn how a plant can alter its growth rate in response to gravity and also how, in general, plants can control and alter their growth rate.

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# PLANT MORPHOGENESIS AND PHYSIOLOGICAL BEHAVIOR IN RELATION TO GRAVITY

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## SUMMARY

Gravity is one of the major environmental factors that influence plant growth. Even so, the basic mechanisms that enable plants to detect, measure quantitatively, and respond to perceived gravitational information are not yet well understood. The objectives of our studies are to learn how earth's gravity and other g forces affect plant growth processes, as well as to better understand how plants grow.

A *Helianthus* Flight Experiment (HEFLEX), which is designed to elucidate the contribution of gravity to the phenomenon of circumnutation, is planned for inclusion on Spacelab-1. An empirical verification test, the HEFLEX Bioengineering Test (HBT), was carried out in preparation for this experiment. The effect of possible alterations in moisture content conditions in microgravity is of particular concern, since shoot height--which shows a correlation with the amplitude and frequency of nutational oscillations--is markedly dependent on soil moisture content. HBT was also a verification test of the hardware and processes planned for HEFLEX.

HBT was the first test of plant growth on the Shuttle. An HBT experiment that was carried out on STS-2 was repeated on STS-3 because the shortened 56-hour duration of STS-2 did not permit sufficient growth to occur. A 1 g control test, with a duration and temperature profile simulating that of the STS-3 experiment, was subsequently carried out.

The objectives of HBT were achieved. There was no significant difference between the trend of the data of shoot height versus soil moisture content, as measured on STS-3 and on the ground. Based on the results, the hardware and the 70% moisture content chosen for HEFLEX are judged to be satisfactory. In 45% of the flight seedlings, one or more roots were exposed above the soil line. (The normal incidence of such exposed roots at 1 g is no more than 1%). There were also a few instances of elevated shoots in which the shoot was "driven" out of the soil as the plant axis elongated. We have recently completed an analysis of this elevated shoot anomaly.

Experiments to determine how quickly the imposition of simulated weightlessness causes damping of circumnutation to minimal amplitude and period have been completed. Another series of

tests, consisting of comparisons of different clinostat configurations simulating specific g-force conditions, are continuing. The goal is to learn whether the biological results of clinostat simulations of particular g conditions are the same; circumnutation is the test parameter.

Our plans include completing the above work. In addition, measurements of the hyponastic response of horizontally clinostatted plants to a range of centrifuge-induced axial g forces will be made. This should help determine the extent to which flexing or flopping of stems contributes to hyponasty/epinasty, since the clinostatted plants should experience much the same flexing irrespective of the presence or absence of the axial force. We also plan to measure the angular displacement of the shoot axis under a range of transverse g loads. This is related to the previous task; the application of transverse g forces to a vertically oriented plant should shed light on the question of sag of the plant axis that occurs during horizontal clinostat rotation. We then plan to determine the magnitude of plant "sag" to be expected at 1 g transverse load--and to translate that information into the expected change of leaf angle (and change in other parameters as well), based on the results of the two experiments just described. Finally, indirect support of the planned HEFLEX (SL-1), GTHRES (SL-4), and FOTRAN (SL-4) flight experiments will continue. The plan of GTHRES is to establish a gravitropic threshold through the delivery of g-pulses, by means of centrifugation, during the flight. FOTRAN will investigate the nature of phototropism in microgravity.

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## AROMATIC BIOSYNTHESIS AND GRAVITY

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### SUMMARY

Gravity is believed to be a primary controlling stimulus for lignification. The approach that has been followed in our investigations of the gravity-lignification relationship has been to elucidate the regulation of aromatic biosynthesis related to lignification in higher plants, to achieve an understanding of the role of lignin and protein synthesis in plant orientation, and to study the effect of near-weightlessness on lignification in higher plants.

Lignin synthesis in young (<10-day-old) pine seedlings responding to geostimulation is not altered in the bend region. Synthesis is increased in the region of the stem below the bend zone, however (this region supports most of the seedling mass, even in a horizontal position). In our experiments, no lignin synthesis gradient across this region has been detected. We have demonstrated a difference in protein content between the upper and lower side of the bend region as a result of geostimulation. No quantitative difference in one-dimensional electrophoresis has been found. Another parameter that distinguishes geostimulation from photostimulation has been successfully separated; bending due to geostimulation is blocked by cyclohexamine whereas photostimulation is not.

The principal accomplishment was the successful flight of a carry-on plant experiment. This was achieved on STS-3 in March, 1982. The specific objectives of this experiment were to test the functioning and effectiveness of a "mini" plant growth unit (PGU), in terms of its ability to support plant growth in space; to observe overall growth and development of young seedlings exposed to near-weightlessness; and to utilize the PGU to investigate the effect of near-weightlessness on the synthesis of lignin.

Thirty-two 4-day-old pine seedlings and 32 oat and 32 mung bean seeds were the starting materials for the experiment. The seeds and seedlings were planted in small chambers which were subsequently inserted into the PGU. The PGU was loaded onto the Shuttle 10 hours prior to launch; a second PGU was prepared as a 1 g control. After the 194-hour flight, the seedlings were observed, measured, photographed, and analyzed.

The experiment demonstrated that the PGUs can be used to support plant growth in space, and that plants can germinate and grow in space (at least during the initial stages). The seedlings appeared healthy but the roots and stems of flight seedlings were shorter than those of the ground controls. Some of the difference, however, was likely due to differences between the two PGUs themselves. Some disorientation was encountered: a substantial number of oat and mung bean roots grew upwards and several mung bean stems grew horizontally; these phenomena were not observed in the ground controls. Neither light nor the water source was adequate to orient all roots down or stems up.

The results of the lignin and other chemical analyses were complicated by the seedling height differences. Expressed on a per cm basis, lignin content of flight seedlings was some 4-8% greater than for ground control seedlings. In pine and mung bean, the differences in lignin content decreased in the upper parts of the stems. There may have been a problem with data expression, however. Baseline work has shown that shorter stems generally contain more lignin on a per cm stem length basis, and, as was pointed out, differences in the height of stems of flight and control seedlings were found. It may be more accurate, therefore, to express the data on a per stem basis. On a per stem basis, total lignin content of flight stems of pine and mung bean was about 4% and 25% less, respectively, than for controls.

The activities of two enzymes in the lignin biosynthetic pathway, phenylalanine ammonia lyase (PAL) and peroxidase, were reduced in space-flown pine seedlings, compared with the controls. PAL activity in flight seedlings was reduced by 15% (per cm basis) and 31% (per stem); peroxidase activity in flight seedlings was 1-2% (per cm) and 22% (per stem) less.

Our continuing studies in this area will focus on searching for specific proteins that are associated with geostimulated bending in pine seedlings and on examining the selective inhibition of cyclohexamide on different bending responses. The search for proteins associated with bending will utilize two-dimensional electrophoresis. The cyclohexamide studies will involve incorporation of radioactive precursors into end products. Attempts will also be made to develop additional analytic tools for the quantitation of early lignification in plant cell walls. Among the analytical methods under consideration is a possible monoclonal antibody-type probe.

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## THE ROLE OF ACID AND CALCIUM GRADIENTS IN GRAVITROPISM

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### SUMMARY

Research on this project is directed toward the understanding of the influence of gravity (or lack of gravity) on plant growth--in particular, the mechanism by which plant roots orient themselves in a gravitational field and grow perpendicular to the direction of gravity, a phenomenon known as gravitropism. During 1982 research was focused on several major questions: (1) What is the role of the two plant growth hormones, auxin and abscisic acid, in the response of roots to gravity? (2) What is the time course of plant responses to gravity and what does this tell us about the response mechanisms? and (3) What roles do secondary growth factors such as hydrogen ions and calcium play in the response of roots to gravity?

In order to facilitate the study of these problems, three methods of recording gravity effects on plant growth with high precision were devised: (1) a highly sensitive "root auxanometer" which allows a computer to record the straight growth of roots continuously with resolution sufficient to detect growth over a period of a few seconds, (2) a special plant growth chamber in which color time-lapse movies of plant responses to gravity can be made for frame-by-frame analysis, and (3) a computer-operated video camera which allows computer analysis of surface extension in plant roots responding to gravity.

Using this specially developed equipment, substantial progress was made in studying the three major questions posed above (hormone involvement, growth kinetics, and roles of protons and calcium). In a detailed study of the comparative effects of auxin and abscisic acid on root growth, it was learned that the effects of auxin are closely parallel to effects to be expected of a gravitropism-mediating hormone. The effects of abscisic acid, by contrast, are not. This is a major finding since it greatly reduces the number of viable models of gravitropism and focuses research along more promising lines.

Using time-lapse cinematography and computer analysis of root surface extension, it was learned that gravitropic curvature is initiated within 15 minutes by enhanced growth on the top and reduced growth on the bottom of the roots. This information is crucial to an assessment of physiological changes in top and bottom cells which might lead to coordination of root responses to gravity.



Among the most striking results obtained in 1982 are the findings that asymmetric gradients of protons and calcium ions parallel precisely the asymmetric growth patterns responsible for root gravitropism. This research has shown a close correlation between proton efflux and growth rates in growing root cells, and has shown that this proton efflux is under the control of the plant growth hormone, auxin. In gravity-stimulated roots there is enhanced proton efflux on the rapidly growing upper cells, indicating a close link between auxin redistribution, asymmetric proton efflux, and gravitropic curvature. This is an especially significant finding, since it ties the action of the plant hormone auxin to the apparent immediate cause of asymmetric growth during gravitropism, i.e., asymmetric efflux of protons from the roots.

In studies begun toward the end of 1982, it was learned that gravitropismlike curvatures can be induced in roots without gravity stimulation by simply applying a calcium gradient at the tip of the root. Additionally, it was found that calcium binding agents applied to the root tip cause the root to lose its sensitivity to gravity. This strongly suggests that internal calcium gradients play a key role in either the detection of gravity or the early response to gravity. This notion is also supported by other recent studies of calcium and gravitropism. This is perhaps the project's most important finding during the past year. It provides, for the first time, a possible physiological tip asymmetry which may mediate the action of gravity on root growth. This lab can induce gravitropismlike curvature without gravity stimulation, and this ability should be invaluable in testing the involvement of the other physiological parameters under study (especially auxin redistribution and proton efflux asymmetry) in the normal control of growth by gravity.

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## GEOTROPIC STIMULUS PRODUCTION IN ROOTS OF CORN

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### SUMMARY

Plants orient their root and shoot axes with respect to the gravitational field.

In roots the gravity stimulus is perceived in a specialized tissue of the root called the root cap. It is hypothesized that once gravity is perceived by the cells of the root cap, a series of biochemical processes occur in the cap and ultimately lead to downward growth in roots. This project is interested in studying the biochemical processes that occur in the cap in the interval of time between the perception of gravity and the downward growth of the root.

For this investigation seedling roots of corn were used. Such roots are particularly suited for this work because if grown in total darkness, roots of corn do not respond to gravity and fail to reorient their axes with respect to the gravitational field. A brief flash of light, however, initiates the bending response. Using light as a trigger for the bending process, this lab has begun to characterize those biochemical processes occurring in the cap that are believed to be involved with translating the perception of gravity into downward growth.

Protein synthesis is necessary for this translation process. If protein synthesis is prevented in the caps, roots fail to bend downward when illuminated. Thus far, work indicates that no new proteins are formed as a result of the light treatment. Rather, the results suggest that several preexisting proteins selectively increase in amount as a result of the light treatment. This would imply that the effect of the light on proteins is quantitative and not qualitative. So if proteins are involved in the translation process we would at present conclude that the gravity translation processes operate in part by selectively enhancing the levels of specific proteins (enzymes?) without affecting the types of proteins present. Currently this lab is using a more sophisticated technique to confirm these preliminary observations, and in particular, to examine the notion that small amounts of new proteins may be produced as a result of the light and/or gravity stimulus.

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## MECHANISMS OF GRAVIPERCEPTION AND TROPISTIC RESPONSE IN PEA SEEDLINGS

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### SUMMARY

In order for a plant cell to sense gravity, it must have some physical mechanism that can convert the energy of a displaced organelle into some signal that will alter the direction of growth. There is considerable evidence that especially dense starch grains, contained in amyloplasts, serve this function in higher plants. Such bodies had never before been isolated intact and clearly identified by chemical markers. This project succeeded in this task in 1982.

Falling amyloplasts exist only in special gravisensitive cells, called statocytes. Using cell-wall digesting enzymes, this lab has succeeded in isolating such cells in a viable condition. When such cells are mounted on a vertical microscope slide, their amyloplasts fall repeatedly to the bottom of the cell as it is rotated. Electrical and chemical studies are planned on these isolated cells to determine the sequence of events following amyloplast displacement.

Samples of oat and mung bean seedlings flown on the third flight of the space shuttle in 1982 in the experiment of J. Cowles were prepared and examined by electron microscopy, making it the first detailed fine structure analysis of space-grown plant material. No anatomical differences were found.

Plants in space are plants under stress. It is thus of interest that the investigators have found a marked rise in the level of putrescine, a common polyamine, shortly after application of various stresses including dehydration, osmotic shock, low pH, high salt, and no potassium. In all cases, the extra putrescine comes from extra, newly synthesized, arginine decarboxylase activity. Investigation of the relevance of this system to gravitational and mechanical displacement is planned.

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## THE ROLE OF GRAVITY IN REGULATION OF LEAF BLADE FORM

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### SUMMARY

Major accomplishments in 1982 have been: (1) the identification of gravity and auxin-sensitive cells in the leaf blade; (2) observations on the role of ethylene, abscisic acid, and other growth factors in regulation of leaf blade form; and (3) characterization of the separable roles of the petiole, pulvinus, and blade in leaf response to gravity.

This lab completed gross anatomical and histological studies and identified specific cell layers in the blade (lower epidermis and vascular bundle sheaths) whose expansion controls the form of the leaf. Radioautographic studies have shown that labeled material applied as the plant hormone indole-acetic acid, or auxin, becomes distributed across the entire blade, consistent with this lab's observation of response in the lower epidermis. Studies of transport of labeled auxin have shown that there is a pronounced dorsiventral gravipositive movement of labeled material but that movement in the opposite direction is minimal.

Ethylene production by leaves given a variety of chemical, physical, and environmental stimuli has been studied. Quantitative analysis of ethylene production throughout the time course of blade response to auxin shows that ethylene production follows blade hyponasty and precedes petiole and pulvinus epinasty. Rotation on a clinostat promotes ethylene production and blade, petiole, and pulvinus epinasty. Treatments that inhibit ethylene production (silver nitrate, aminoethoxy-vinylglycine) inhibit petiole-pulvinus epinasty but not blade curvature. Treatments that do not promote ethylene production (2,3,5-triiodobenzoic acid, Na-N-1-naphthylphthalamic acid, abscisic acid, gibberellic acid) do not promote leaf angle change. Ethylene appears to be required for leaf angle change (petiole-pulvinus epinasty), and auxin appears to be required for blade curvature.

This project is now studying the hypothesis that the blade is the part of the leaf that senses a change in gravitational orientation, and that the system regulating this is auxin transport. The pulvinus is the part of the leaf that responds to a change in gravitational orientation, and the system regulating this is ethylene synthesis. Auxin-induced ethylene synthesis is the link between blade perception and leaf angle response to gravity.



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# EFFECTS OF GRAVITY AND LIGHT ON GROWTH, DEVELOPMENT, AND FLOWERING IN PLANTS: I. EFFECT OF THE CLINOSTAT ENVIRONMENT

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## SUMMARY

A goal of this project is to grow plants in space weightlessness for several successive generations. In doing so, we may better understand how the growth, shape, size, and reproduction in plants are influenced by the force of gravity and whether gravity is essential for the survival of plants.

The most significant finding during 1982 on this project was that mouse-eared cress plants, *Arabidopsis thaliana* (L.) Heynh., were successfully grown through one generation (seed to seed) on the clinostats. Abundant seeds were produced by plants cultured axenically in tubes sealed by urethane foam or cotton plugs. The viability of the seeds produced by plants growing on clinostats is being tested. It is planned to test-grow plants on the clinostat for three successive generations.

In sealed cultures, mouse-eared cress failed to produce seeds in all treatments. In horizontally clinostatted sealed cultures, the roots grew into areas where moisture condensed on the tube walls, and thereby hindered shoot growth.

Plants, as measured by plant height, grew faster in larger containers. These results indicate that the CO<sub>2</sub> supply may be a critical factor in growth and floral development rate, as evidenced by growth in containers having free atmospheric gas exchange. We plan to grow mouse-eared cress in sealed 5-gallon bottles since calculations indicate enough CO<sub>2</sub> is present in the bottle to provide the carbon requirements for a mature plant with seeds.

Three varietal strains were tested for seed-to-seed growth. All three strains failed to set seeds in sealed culture tubes (25 x 200 mm) containing 20 ml of agar medium. Good seed set occurred for all three strains in cultures provided ample gas exchange.

More leaves (15 to 18) and stems (3 or more) were produced by plants under sealed conditions than in open cultures (7 to 10 leaves and 1 or 2 stems). The multiple leaved and stemmed plants failed to set seeds. The prime suspects, high moisture and low CO<sub>2</sub> levels, will be investigated.

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# EFFECT OF GRAVITY AND WEIGHTLESSNESS ON THE DEVELOPMENT OF GIANT COENOCYTE, CAULERPA

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## SUMMARY

The organism used--Caulerpa--is one of the largest cells known to science. Its giant size makes it of particular interest in following what happens inside a cell when gravity responses occur.

Gravity effects on the regeneration of parts of Caulerpa were studied. Segments of "blade" from two species were studied under normal orientation to gravity and inverse orientation. In contrast to earlier findings on the site of rhizoid differentiation on the inverted rhizome, gravity had no effect on the location or number of regenerated organs on these "blade" pieces. Segments of rhizome, however, failed to exhibit a strict polarity of regeneration with respect to gravity. Hence, gravity acts differently or not at all on the different "organs" of this giant cell.

Investigations as to what occurs inside the cell when there is a gravity effect resulted in the discovery that, before gravity causes a relocation of the site of rhizoid formation, it causes microscopic organelles inside the cell to sediment to the lower side of the inverted rhizome. These organelles are amyloplasts, as revealed by histochemical staining and polarized light studies. Such sedimentation of amyloplasts occurs in just the sites where rhizoids will later form, further confirming that they are the cause of the relocation of rhizoid formation.

Based on the hypothesis that the amyloplasts act via hormones, two known hormones from multicellular plants have been investigated. Treatment of the organism with gibberellic acid increased rhizome elongation and caused more frequent formation of rhizoids, thus making this hormone a likely candidate for the mediating hormone in rhizoid relocation under gravity.

The other hormone that was apt to be involved was indole-3-acetic acid (IAA). This hormone was not believed to be present in Caulerpa, from earlier work by others, but by applying improved techniques it was demonstrated by both bioassay and electrochemical detection after High Power Liquid Chromatography (HPLC) that IAA was present and in sizable amounts. Its action on Caulerpa is to stimulate "blade" growth, so it seems to act in concert with gibberellic acid to coordinate the development of the giant cell.

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## MECHANISM OF THIGMOMORPHOGENESIS IN PLANTS, AND ITS SIGNIFICANCE TO RESEARCH WITH PLANTS IN SPACE

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### SUMMARY

One of the most important accomplishments of 1982 was developing the computer-assisted image processing instrumentation to the point where it is on-line and being used in experiments. It has taken three years to reach this point. This instrument uses a video camera as the "eye". The image produced by the camera can be enhanced by electronic means so that it is possible to see on the video monitor features in a subject which cannot be otherwise visualized. By linking the image electronically with a microcomputer, and writing special programs for the computer, the instrument will scan the image, generate data, and plot out computations based on the data. For example, the camera can be pointed at a plant stem bending upward during gravitropism, scan the image at selected intervals, and the computer will automatically draw graphs representing the changes in curvature and length of the upper, lower and midline parts of the stem with time. Using another program, it is possible to automatically count, measure the size of and the average density of objects such as cells under a microscope, birds in a flock, or trees in a forest. This instrument is currently being used in several projects of interest to NASA.

This laboratory has previously shown that the plant hormone ethylene is responsible for the thickening of plant stems that have undergone mechanical stress or perturbation; current evidence strongly suggests that when the plant is stressed, it produces a compound called an "elicitor" which then induces the production of ethylene. The elicitor has not yet been identified, but it may be of considerable importance in plant responses to changes in the environment.

When a plant is mechanically stressed or gravitationally stimulated, it is found to produce large amounts of a special kind of cell wall material called callose. This callose, when measured under the microscope with the computer-assisted video processor, may also be involved in the stimulation of the production of ethylene. At this stage, no one knows how this happens, but this laboratory is planning experiments to shed light on this important problem.

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# INVESTIGATION OF THE NEGATIVE GEOTROPIC RESPONSE IN SHOOTS OF THE OAT PLANT (Avena sativa)

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## SUMMARY

Since cereal grasses are our most important source of food, we need to know more about the gravity responses of these plants. This knowledge is essential for growing cereal plants in outer space as well as in improving their yield on this planet. The most important gravitropic organ of cereal shoots is the pulvinus. This organ keeps the plants erect and allows the grains to develop well above ground level. Failure of this organ would lead to grain losses of severe consequences. Knowledge gained from this study of the gravitropic response in the grass pulvinus will have equal applicability to other plant organs involved in gravitropism.

The overall objective of this project is to shed light on how the gravitropic stimulus is perceived by gravisensitive leaf-sheath pulvini of grass shoots when they are subjected to a gravitropic stimulus--especially the nature of the starch statolith-cell membrane interaction that we have observed in this system.

A model has been formulated which explains in one simple equation the individual response of every cell in an organ that is responding to gravity. Important inferences from the model include: (1) a fundamental distinction between the gravitropic responses of multicellular and unicellular structures, (2) cellular asymmetry as a basis for organ asymmetry, and (3) difficulties in accepting lateral transport of hormone as a basis for gravitropic response in plants.

During the perception phase of the gravitropic response in the grass leaf-sheath pulvinis, it was found that the vacuolar membrane (the tonoplast) interacts with the organelles which sediment in response to gravistimulation, namely, the starch statoliths. The tonoplast surrounds each starch statolith at the bottom of each statocyte (statocyte = a cell containing statoliths). This is the first direct proof for starch statolith interaction with a cellular component, here, the tonoplast membrane.

It was demonstrated that in barley, the starch statoliths show intense esterase activity when stained with fluorescein diacetate. Such esterase increases on the lower side of a gravistimulated pulvinus in comparison with the upper side; staining with naphthol As-D also confirms the increase in esterase activity on the lower side of gravistimulated pulvini.



The cellulose synthesis inhibitor, DCBN (2,6-dichloro-benzonitrile), significantly blocks gravitropic curvature in gravistimulated grass leaf-sheath pulvini, and at the same time, causes large thickenings to appear in the radial walls of pulvinus cells, first in the statenchyma, then in other cells, and with increasing frequency as one progresses from top to bottom of the gravistimulated pulvinus. Their distribution pattern closely follows the equation that describes the geometry of asymmetric growth in the leaf-sheath pulvinus.

With the use of continuously recording transducers, it was shown that corn seedling coleoptile responds to gravistimulation within 15 minutes, and curves upward at the rate of  $1^\circ$  per minute until the coleoptile is vertical at 120 minutes. In systems with barley and oats, the pulvini start to curve upward within 20 to 30 minutes after they are gravistimulated, but here, the rate of curvature is much slower, on the order of  $1.5^\circ$  per hour for a total period of about 48 hours.

Collections were made of tops and bottoms of gravistimulated and "lefts" and "rights" of upright pulvini at different times during the course of gravitropic curvature in oat and barley pulvini for cell-wall analyses; these wall polysaccharide analyses (cellulose, pectins, hemicellulose) are currently in progress.

Also, collections of similar fractions of corn at different times after feeding the pulvini with  $^3\text{H-GA}^{20}$  (gibberellin  $\text{A}^{20}$ , labeled with tritium) to follow the time-course changes in GA metabolites were carried out; these analyses are currently in progress. Earlier studies of oats showed that GA conjugates were at their highest levels in the upper halves, and the free active GAs ( $\text{GA}_3$ ,  $\text{GA}_4$ ,  $\text{GA}_7$ ) were at their highest levels in the lower halves of gravistimulated pulvini.

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## CELLS, EMBRYOS, AND DEVELOPMENT IN SPACE--MORPHOLOGY OF PLANT CELLS IN SPACE

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### SUMMARY

The ultimate overall objective of this research project is to ascertain whether higher plants can carry out their full development, reproduction, and growth in near-0 G despite the fact that their evolution occurred under the asymmetric 1 G conditions of Earth.

Experimental systems are being developed at different developmental or organizational levels to determine what a minimum gravitationally responsive organizational unit will be in space. Cells which have had their cellulose walls removed (protoplasts) and free cells which derive from nonsexual body cells (that can nevertheless behave as if they were embryos derived from the fusion of sex cells) have been the subject of our special attention, because these represent test systems that can develop into entire plants even as they "bypass" the sexual process. Simultaneously, investigations on the growing points or zones of focused cell division and growth are made since these represent a much higher degree of organization than that of isolated, free cells and protoplasts. Cell theory states that cells are the units of life, but it has not been established whether higher plant cells in isolation act as receptors of gravity. Morphological complexity, as in a discrete growing shoot or root tip, may be required.

The hope is that by using test systems at different levels of initial organization, but which are capable of attaining the most advanced degrees or levels of higher plant shape and form under closely controlled environments, research will determine whether highly asymmetric growing regions of shoot and root are really crucial to detectable degrees of responsiveness. Moreover, since nutrition and metabolism of higher plants supports and are mediated by highly asymmetric systems such as shoot and root, it is considered critical to learn whether all these events and subsequent physiological functions can be established and if they can be established repetitively under hypogravity conditions and can proceed virtually continuously in the near weightless state to establish consecutive multiple generations. This will disclose whether there is any remnant or a retention of a sort of "memory" of the gravity experienced on Earth.

Significant progress has been made in initiating non-sex-cell suspension cultures of a number of higher plants which have been carefully selected because they represent different kinds of growth habits. Each of these can go on and organize into plantlets as they mimic the events of sexual embryo formation and growth. Recent progress has not only been made in increasing the speed with which the embryonic structures are induced, but in the degree of precision with which they undergo development.

Since cloning of higher plants via cells and protoplasts presumes genetic fidelity, we have recently developed methods that permit chromosome analysis at the cell and protoplast level with more precision than has been possible before. This methodology will help to establish decisively whether cells and protoplasts exposed to various "insults" respond by visible changes in their karyotype or chromosomal profile.

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## THE AMYLOPLAST AS A GRAVITY-SENSING DEVICE IN PLANTS

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### SUMMARY

This study concerns the early events in the plant's perception of gravity. Gravity-sensing plant cells contain starch-filled organelles (amyloplasts) which settle under the influence of gravity. The movement of these organelles somehow results in the establishment of a physiological gradient across the plant organ which ultimately results in the curvature of the organ (e.g., root, stem, or coleoptile) toward or away from gravity.

It is not known how amyloplast movement causes this differential growth. This laboratory has taken two approaches to uncover what this connection may be. The first was to try to characterize the surface properties of these organelles. Intact amyloplasts were successfully isolated from gravisensitive tissues. Their intactness was confirmed with the electron microscope.

This isolation enabled a determination of whether amyloplasts possess a strong surface charge. Some workers have proposed that if amyloplasts were electrically charged, their settling could create an intracellular charge redistribution that may play a role in gravity perception. To find out, amyloplasts were placed in a small glass cell and exposed to an electric field while being observed under the light microscope. By measuring the rate and direction of their movement, it was discovered that there is a significant net negative surface charge on these organelles. This lends some support to the charge redistribution hypothesis although it does not directly answer the question of whether the amyloplast charge plays a role in gravity sensing.

Using various techniques, including an ion microscope and a fluorescent indicator (the antibiotic, chlorotetracycline), it was also determined that amyloplasts contain a substantial amount of calcium. Some of this calcium is bound to the charge shell that surrounds the organelle. Since experiments from other laboratories indicate that calcium may play a role in the plant's response to gravity, it is possible that the settling of amyloplasts redistributes calcium intracellularly, perhaps in a manner useful in gravity perception.

A second approach to studying gravity perception involves the examination of the response of living cells to gravity while they are being observed with the light microscope. Very little is known about what occurs in living cells when amyloplasts

sediment. Most of the information is derived from studies of tissues fixed (preserved in a dead state) for light and electron microscopy. This laboratory is studying the process of sedimentation in living cells by cutting thin sections of plant organs and observing the cells under a microscope that is horizontally mounted. Rotation of the microscope stage results in a changed position of the cells and their parts with respect to gravity.

Many of the hypotheses purporting to explain the phenomenon of plant gravity perception are based on studies of sedimentation after the first few minutes. However, recently it has been determined that it takes the plant less than a minute to perceive the new gravity stimulus. Thus, it becomes particularly important to determine the path and rate of movement of the amyloplasts during this initial period.

Many scientists think of the structure of the cells that perceive gravity in fairly static terms. It is not widely known that the contents of many of these cells are under constant internal motion. The cytoplasm in healthy graviperceptive cells undergoes active and multidirectional streaming. Very little is known about how this streaming itself responds to gravity or of how streaming affects the rate and direction of amyloplast movement. This laboratory is in the process of assembling a high resolution system that links a video camera and recorder to a light microscope so that these questions (and others) can be answered.

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## MECHANICAL REGULATION OF PLANT GROWTH AND DEVELOPMENT

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### SUMMARY

During 1982, activities in this laboratory have emphasized effects of periodic mechanical shaking or vibration on the photosynthetic productivity of one plant species, soybean (Glycine max L. cv. Wells II). The research of this laboratory as well as that of others has shown that these same mechanical forces have an inhibitory effect on plant growth and development on Earth, the main natural counterpart of which is represented by wind. In space, however, mechanical vibrations are measured in the same units as gravity; i.e., they have "g" equivalents. Thus, it is imperative that well-controlled flight experiments be conducted to determine positive as well as negative aspects of mechanical action on plants in an environment otherwise deprived of mechanical stimuli.

Growth analysis studies with soybean in this laboratory indicate that overall photosynthetic productivity of soybean (measured as whole plant dry weight) is sharply reduced if the plants are agitated for 3- to 4-minutes two or three times daily on an orbital shaker. This means that not only is biomass accumulation affected by mechanical disturbance, but so also is air revitalization ( $O_2$  generation,  $CO_2$  scavenging) capacity. During 1982 this laboratory has conducted a number of mechanical stress experiments in controlled environments involving growth dynamics analysis. This approach involves sequential harvest of treated plants over time and the calculation of growth dynamics indices such as relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR). RGR is the slope of the log-transformed cumulative growth curve (measured as whole plant dry weight) vs. time during exponential growth, and is related to the other two growth index parameters by  $RGR = NAR \times LAR$ . NAR is a measure of photosynthetic efficiency and LAR is the ratio of leaf area to whole plant dry weight. One of the most significant findings during the past year was that RGR of mechanically stressed plants was less than that of undisturbed control plants. Furthermore, analysis of RGR components indicated that reduction in NAR accounted for virtually all of the decline in RGR, whereas LAR either was unaffected or actually increased.

Further investigations of possible causes of stress-induced decreases in NAR by means of leaf porometry and measurements of whole plant transpiration and leaf water potential have been



made. Each of these three measurements reflect plant water status, which in turn may reflect the degree of opening or closing of stomatal pores on the leaves. Briefly shaken plants transpired significantly less water vapor out of their foliage for at least 90 minutes following a single 3-minute shaking treatment. Preliminary leaf water potential measurements indicate that 30 minutes after shaking a soybean plant, both leaf and stem water potential are higher for shaken plants than for undisturbed control plants. All of these measurements are consistent with the interpretation that shaking causes a temporary closure of the stomatal pores, thereby lowering transpirational water loss and causing improvement in the water status of stems and leaf cells.

Future efforts will emphasize measurement of leaf gas-exchange rates as a function of mechanical stress pretreatment, which will involve direct measurement of photosynthetic CO<sub>2</sub> fixation and determination of component resistances contributing to the suspected overall increase in leaf resistance to CO<sub>2</sub> fixation.

Complete characterization and analysis of gas-exchange responses to mechanical disturbance are very important for understanding the impact of such forces on the vital life support functions (i.e., primary productivity) of plants. This approach eventually must be extended to the weightless or hypogravity situation to appreciate its full importance in an orbiting spacecraft.

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## HOW THE PEA STEM SENSES GRAVITY, FRICTION, AND FLEXURE

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### SUMMARY

This is a new project. It is proposed that stimulation of cells in the pea stem by gravitational and other acceleration, by friction, and by flexure results in delicate deformation of the plasmalemma. Consequent deformational shifts in macromolecules controlling the permeability of the membrane for calcium ion result in its movement into the cytosol down an actively maintained concentration gradient. As the amount of cytosolic calcium rises, it binds to the modulator protein calmodulin, which in turn can phosphorylatively activate (1) a membrane-bound protein which transports the hormone indoleacetic acid out of the cell, and (2) a tubulin-type protein which, upon polymerization, brings about exocytosis of vesicles containing an agent that can stimulate ethylene production in surrounding cells. Because gravitational acceleration is vectorial, the consequent vectorial activation of auxin transport enzyme ultimately leads to an asymmetry of the growth hormone across the stem and geotropic curvature results. Because friction and flexure do not tend to distort membranes with net asymmetry, and because ethylene is a rapidly diffusing molecule, these stimuli symmetrically result in the "slower" sturdier growth of the stem which ethylene induces. To the extent that activation of the two types of protein overlaps, the responses interfere with each other; in particular, since ethylene tends to antagonize auxin effects, this explains the prominent counterreactive phase of geotropism.

Using  $^{32}\text{P}$ , phosphorylation of proteins will be studied during and following stimulation by gravity, friction, and flexure. Any phosphorylated protein increased by the stimulation will be isolated so that biochemical properties may be studied.

Voltage transients that may represent vesicle release have been described following stimulation, and will be subjected to more rigorous study. Conditions governing their frequency will be evaluated.

The putative agent released from the vesicles for stimulation of ethylene production will be sought.

The release of ethylene will be monitored at a fine level of discrimination during and following gravitational stimulation, looking for correlation between the frequency of voltage transients, the abundance of this gaseous hormone, and the counterreactive phase of geotropism.

## MECHANISM OF GRAVITROPIC CURVATURE IN PLANT SHOOTS

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### SUMMARY

A basic but as yet unresolved problem in plant physiology is the mechanism by which plants transduce information about the direction of gravity into an oriented direction of growth. During the past 2 years (1981-1982), this project has focused on this problem using the gravitropic curvature of plant shoots as a model.

Briefly, it is known that when a shoot is oriented in a horizontal position it begins to curve upward in a smooth arc after about 30 minutes. Reorientation is complete within 2 to 4 hours. This curvature response ultimately derives from enhanced growth of those cells comprising the lower half of a horizontal shoot and a retardation of cell growth near the upper surface. This research centers on the factor or factors which are responsible for such asymmetric growth.

In 1981, Rayle reported that a proton gradient develops across gravitropically stimulated shoots, and that this gradient is critical in initiating the asymmetric growth that gives rise to negative shoot gravitropism (i.e., upward curvature). Research during 1982 has been directed toward sorting out whether graviperception mechanisms give rise to the proton gradient directly or whether the proton gradient is derived indirectly, perhaps as a result of auxin (indole-3-acetic acid; IAA) redistribution. The latter possibility would be consistent with much existing data, while the former possibility, if correct, would be a radical departure from classical thinking on this subject but consistent with the chemiosmotic theory of auxin transport. The data described below represent our approach to resolving these alternatives.

Using  $^3\text{H}$ -IAA, this investigation has established that auxin redistribution occurs within 30 minutes of gravistimulation. This redistribution occurs simultaneously and uniformly along the growing axis. These data suggest the kinetics of auxin redistribution are rapid enough to directly initiate asymmetric proton secretion.

High molarity neutral buffers prevent shoot gravitropism by neutralizing excreted protons and thus prevent an acid asymmetry from developing across a shoot. However, such buffers do not prevent development of a  $^3\text{H}$ -IAA asymmetry. These data thus show a proton gradient is not necessary for IAA redistribution.

Rather, they are consistent with the notion that IAA redistribution precedes asymmetric proton secretion and may in fact initiate the proton gradient.

The fact that the lateral auxin redistribution appeared to be qualitatively and quantitatively normal when shoots were submerged in neutral buffers was surprising. Purely theoretical considerations which have been formalized as the chemiosmotic theory of auxin transport would predict otherwise. In an attempt to explain this paradox, the investigators have begun experimentally probing the chemiosmotic theory.

This line of experimentation involves altering the cell wall pH of shoots via infiltration with buffers and/or by treating shoots with agents that inhibit (e.g., cycloheximide) or stimulate (e.g., fusicoccin) in vivo H<sup>+</sup> secretion. Preliminary results do not support the chemiosmotic theory. Lateral and polar auxin transport are not influenced by cell-wall pH.

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## THE CELLULAR BASES OF GRAVITY- AND LIGHT-INDUCED GEOTROPISM

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### SUMMARY

The overall objective of this research project is to arrive at a better understanding of the specific fundamental processes that are altered by gravity and light to produce geotropic growth in plants.

Previous results from this and other laboratories give reason to believe that at least one of these fundamental processes may be the movement of calcium (Ca) ions in the stimulated cells and organs. These results indicate that a gravity stimulus promotes the asymmetric distribution of Ca across growing stems. Light activation also promotes calcium movements in plants. Calcium is known to reduce the plasticity of plant cell walls, i.e., reduces their ability to be stretched in response to a force, such as intracellular turgor pressure. After a plant has been given a new gravity stimulus by turning it to a horizontal position, higher concentrations of Ca accumulate in the walls of cells on the upper side of its stem. Cells on this side will grow more slowly than those on the lower side as the stem curves upward in response to the gravity stimulus. It has been proposed that Ca, through its effects on wall plasticity, could play a role in mediating this differential growth. This project is focused on examining whether Ca does play an important role in mediating geotropism and, if so, how it does so.

Experiments during 1982 further examined the Ca hypothesis by testing inhibitors which would be expected to interfere with Ca accumulation in walls and by testing whether Ca redistribution could also be observed in roots as it had been observed previously in shoots. The major findings are as follows:

1. Correlation between the amount of  $^{14}\text{C}$ -chlorpromazine (CPZ) bound to calmodulin (CaM) in oat coleoptiles and how effectively CPZ blocks geotropism in coleoptiles.

The regulatory protein CaM, controls the activity of a plasma-membrane-localized ATPase in plants which serves to pump calcium out of cells. This pump probably plays an important role in the asymmetric movement of Ca which has been postulated to be one of the early transduction steps necessary for geotropism. CPZ, a CaM antagonist, reversibly inhibits the calcium pump. It also inhibits geotropism in oat coleoptiles at concentrations that permit normal growth rates. This lab used  $^{14}\text{C}$ -labeled CPZ

to photoaffinity label CaM in vivo to determine if the drug is actually binding to some portion of endogenous CaM when it inhibits geotropism. It was found that CPZ does bind to cellular CaM in coleoptiles when it blocks their geotropism and that those coleoptiles that showed the least geotropism after CPZ treatment had the greatest amount of their CaM bound to CPZ. This correlation is consistent with the hypothesis that CPZ blocks geotropism at least in part through its blockage of the CaM function.

## 2. The calcium chelator, EGTA, blocks geotropism in oat coleoptiles.

The chemical EGTA strongly binds, or chelates, Ca and it is very effective in removing Ca from walls of plant cells. It was found that perfusion of oat coleoptiles in 1 mM EGTA for 6 hours or more blocks geotropism in 60% of the coleoptiles (compared with blockage of only 10% of coleoptiles perfused in water), but does not inhibit growth of these coleoptiles. These results support the hypothesis that the accumulation of Ca in walls of cells in geotropically stimulated coleoptiles helps to mediate their tropic growth.

## 3. Rapid structural changes occur in the walls of epidermal root cells after geostimulation.

In preliminary ultrastructural studies, we did not observe an asymmetric buildup of Ca in the walls of geostimulated root cells, but we did detect a rapid (<30 minute) change in the appearance of the outer walls of epidermal cells on the upper (faster growing) sides of the roots. These walls appear less dense and to be minus a thin outer layer present on the outer walls of epidermal cells on the lower sides of the roots. These results indicate that rapid chemical changes are occurring in the upper epidermal walls, and tests are underway to determine what they are and how they relate to geotropism.

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# AN EXAMINATION OF THE CLINOSTAT, AS A SIMULATOR OF WEIGHTLESSNESS USING LEAFY PLANTS

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## SUMMARY

A weightless condition may be simulated in the lab by using clinostats to rotate plants about a horizontal axis, or by periodic inversion. During the past few years this laboratory has developed several treatments to separate plant responses caused by mechanical stresses, during clinostatting, from those effects caused by gravity compensation. Several parameters were measured, including (1) height to a point marked near the apex of the stem, (2) height to a point about two internodes below the above point, (3) stem diameters at the two marked points, and (4) leaf epinasty.

Changes in stem heights and diameters showed no simple patterns among the various treatments nor for the two species used in our studies: cocklebur (Xanthium strumarium) and tomato (Lycopersicon esculentum). For example, stem elongation (especially of young internodes) was inhibited by mechanical stress and gravity compensation in cockleburs but promoted in tomatoes. It may be safe to generalize that both gravity compensation and mechanical stress typically inhibit, but may sometimes produce, stem elongation. Gravity compensation may be especially important in promoting elongation of older, more mature stems, and this could be related to the stimulated elongation of grass nodes induced by clinostatting.

Studies have also been made of the mechanics of stem bending by placing plants in a horizontal position and wrapping threads around a wire frame so that they are unable to bend upward in response to gravity. Changes in surface dimensions and internal pressures of such plants were then measured before and after release from restraint (at which time they spring to a strongly bent position), and also in vertical controls and plants laid horizontally and allowed to bend freely. When the restrained plants were released, stretching of bottom surfaces and shrinkage of top surfaces was found, and pressures produced by upward bending continued to increase until the time when maximum bending upon release had been reached. Plants that were released from restraint, then forcibly straightened and restrained again, continued to develop bending forces.

Another experiment involved slicing cocklebur or castor bean



(Ricinus communis) stems. Plants were placed horizontally and were allowed to bend freely or were restrained for 48 hours in the dark. They were: (1) cut longitudinally, either parallel or perpendicular to the bend; or (2) small cuts were made at right angles to the stem along the top or the bottom of stems. Vertical control stems were also cut in these experiments. The results clearly showed that the surface layers of stems are under tension compared with the inside, and also suggested that the pressures may be located in the cortex and vascular tissues rather than in the pith. Plants placed horizontally and allowed to bend freely then slit through the center with a horizontal razor blade showed strong bending in the upper half, but little or no bending in the lower half. This again shows the tension in the outer layers compared with pressures in the inner tissues, and emphasizes the importance of the response in the top half of the stem in producing normal gravitropic bending.

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## SPECIAL PROJECTS



## RESEARCH IN SPACE BIOLOGY

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### SUMMARY

Within the next decade, the NASA Shuttle Program will provide a unique opportunity to conduct biological research in outer space and to continue relevant ground-based space biology research. To maximize this potential, there is a need to develop a growing cadre of scientists who are knowledgeable and interested in space biology. The purpose of this grant is to provide NASA Research Associate Awards to young scientists who are interested in working in space biology. These are competitive grants. It is anticipated that these scientists will develop their research careers in the newly evolving discipline of space biology.

The Research Associate Awards are for a 1-year period of funding with the possibility of renewal for a 2nd year of additional funding. Since the program's inception on June 1, 1980, 24 awards have been made. So far, 8 of the 16 scientists selected have received a 2nd year of funding.

The award recipients work in NASA-funded laboratories or in laboratories that can provide the necessary facilities and environment for specialized space biology projects. In June 1980, 19 laboratories participated in this program; as of February 1983, 42 laboratories are now participating.

The grant is administered through the University of Louisville, Louisville, Kentucky. Dr. X.J. Musacchia, Dean of the Graduate School, is the Project Director and Science Advisor.

In addition to the salary stipend, travel expenses are paid for the awardees to attend and present papers at two national meetings: (1) the annual AIBS/NASA meeting, and (2) a national society meeting of their choice.



## MEETINGS





## MEETINGS

Organized or participation by the Space Biology Program

"AIBS/NASA Space Biology Panel Meeting," John F. Kennedy Space Center, Kennedy Space Center, FL, March 1982.

"XXIV COSPAR Meeting," Ottawa, Canada, May 16-June 2, 1982.

"Workshop on Plant Growth in Space," Federation of American Societies for Experimental Biology, Bethesda, MD, May 20-21, 1982.

"Workshop on the Regulatory Functions of Calcium and the Potential Role of Calcium in Mediating Gravitational Responses in Cells and Tissues," Federation of American Societies for Experimental Biology, Bethesda, MD, September 16-18, 1982.

"33rd Annual Fall Meeting of the American Physiological Society," San Diego, CA, October 10-15, 1982.

"Fourth Annual Meeting of the IUPS Commission on Gravitational Physiology," San Diego, CA, October 10-15, 1982.

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